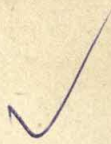


# ALGAL CELL BIOLOGY

SECOND EDITION

H. D. KUMAR



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SECOND EDITION

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# Preface

Faced with the problems of increasing population, poverty, protein deficiency, and pollution, many developing countries are evaluating the potential of freshwater microalgae and marine macroalgae in solving the above problems of the 4 P's; these algae have served as human food both in times of famine and in times of plenty (as, for instance, in Japan). Some of these algae being veritable protein concentrates can constitute the ideal food for vegetarians. They concentrate metal and other ions from the ambient medium and can make up mineral deficiencies. A large number of projects are now underway to mass-culture *Spirulina* and to use the resulting biomass as single-cell protein either for human consumption or for incorporating in the feed rations of poultry and fisheries.

Because of these and other reasons, increasing research effort is now devoted to the study of diverse algae. In this book, I have attempted to describe the fine structure of algal cells and cell organelles in the light of recent researches using the electron microscopic and other related techniques. These techniques have opened up new vistas undreamt of by F.E. Fritsch and other classical phycologists, and an understanding of the fine structure of algal cells has not only advanced our knowledge of the micromorphology, cytology, and genetics of these important primary producers, but has also facilitated meaningful correlations between the structure and function of cells and their organelles.

The term "biology" (used in the title) connotes a wide area that is impossible to cover in a small monograph. I have used this term mostly in the sense of fine structure and cytology, with some physiology, biochemistry, and genetics interspersed here and there. Thus, whereas chloroplasts are described in some detail, pigments are covered only briefly. The increasingly important role of cyanobacteria in biotechnology, especially hydrogen production and nitrogen fixation, is described in Chapter 1.

Some instances of recent additions to our knowledge of algal cell biology include the analysis and mapping of some chloroplast genomes and the vast information on eyespots, flagella, and cell membranes. These and other related aspects are covered concisely in this monograph.

The electron microscopic observations of algae have often raised several new questions pertaining to their relationships, classification, and phylogeny. I have attempted to discuss these wherever appropriate.

By far the most valuable help in the preparation of this monograph was rendered by Professor K. Ueda of Nara Women's University with whom I had the pleasant privilege of working during 1981-82. He also very kindly provided several electron micrographs. I am deeply obliged to him for his hospitality, understanding, encouragement, and affection. Continuing help from him in the improvement and revision of this book is also greatly appreciated.

I am thankful to the Japan Society for Promotion of Science for giving me the opportunity to spend one of the happiest and most memorable years of my life in the wonderful land of the rising sun, amidst the kindest people in the world. It was during this period that much of the first edition of this book was completed.

I am much obliged to Dr. M. Lefort-Tran (Gif-sur-Yvette, France) who was



very generous in giving several excellent electron micrographs. I also thank Drs. E. Gantt, D.J. Hibberd, J.D. Dodge, T. Vaara, S.P. Gibbs, and B. Galatis for providing micrographs, reprints or valuable suggestions. I am extremely indebted to Professor W. Nultsch of Philipps Universität, Marburg (FRG) for his kind help, advice, encouragement, and hospitality during 1983-84 and again in 1988, when I was a Guest Professor in his laboratory where I undertook some revision and updating of this monograph.

H.D. Kumar



# 1 General Introduction

## WHAT ARE ALGAE?

The algae comprise a large, varied, and heterogeneous group of organisms with enormous diversity of form, structure, reproduction, and life history (Fritsch, 1935, 1945). They are relatively simple plants whose ecological, biochemical, and physiological attributes are equally wide and varied so much so that, as a group, they have little in common except their characteristic oxygen-evolving type of photosynthesis. "Algae" is a general term to cover an assemblage of phylogenetically and taxonomically unrelated groups of lower, pigmented plants. It is not a taxonomic but a structural and functional term, and does not necessarily imply phylogenetic relationships among the organisms included.

The algae are mainly defined by: (1) their simple reproductive organs (whether spore- or gamete-producing), which are not enveloped by any wall of sterile cells and (2) their zygotes not developing into a multicellular embryo while still contained within the female reproductive organs. These two characters are also shared by fungi but, unlike algae, the fungi lack photosynthetic pigments as well as photosynthesis.

Being photosynthetic, algae occur wherever there is some light and moisture. Though found in diverse habitats, most algae are aquatic. They occur in freshwaters of all kinds and, in the sea, they occur on water surface as well as in coastal areas. The marine microalgae and seaweeds probably collectively comprise the largest biomass of plant matter on the surface of the earth. Algal cells contain relatively little structural material and most of their body has potentially nutritious or other economic value. Unlike conventional crops, algae are advantageous in improving the efficiency of solar energy utilization for valuable biological products. For this reason, serious attempts are being made in several countries to grow algae as energy crops, for production of biofuels, as source of single cell protein, and as sources of diverse economically- and pharmaceutically-useful products. The commercial feasibility of algal mariculture has been amply demonstrated in Japan which has perfected efficient biotechnology for *Porphyra* farming along the seacoast. Japan also leads the world in successful industrialization of edible *Chlorella*.

Algae are primary producers and contribute to the productivity of fisheries. By growing in polluted water, they glean nutrients which can be harvested as algal biomass and, after appropriate treatments, incorporated into animal feeds. At the same time, the polluted water is reclaimed in a partly purified state. Apart from their usefulness, some algae have much nuisance value as they can cause obnoxious water blooms, degrade water quality, and can be toxic to animals and humans.

Historically, the larger seaweeds were the first to attract the attention of naturalists and herbalists some three centuries ago. These, and the smaller forms (both freshwater and marine) were investigated by means of the light microscope in the nineteenth and twentieth centuries, and a wealth of useful information about their structure and reproduction has accumulated. With the advent of electron microscopy, many different algal taxa have been examined under the electron



microscope during the last few decades and more than 2000 research papers have been published in which some aspect of algal cytology or ultrastructure has been described. The scanning electron microscope has revealed several hitherto unknown structures in diverse algae, especially diatoms and desmids, during the last decade. *In situ* investigations of periphyton by scanning microscopy have shed valuable light on community structure, competition, and succession. We know the reproductive stages of many algae much better now, thanks to scanning electron microscopy (SEM). In fact, electron microscopy [both SEM and TEM (transmission electron microscopy)] has catalyzed a rapid increase in our knowledge of the algae during the last three decades, each new piece of information sometime contradicting previous data and necessitating constant rethinking. This information explosion has shown that the views of classical phycologists, to the effect that algae could be classified into a few phyla, may no longer be tenable. Several schemes of modern, primary classification of the algae have been proposed in the era of electron microscopy and the number of classes, or even phyla, has tended to increase, but so far there is no final consensus as to the superiority of one system over another. Algal ultrastructure and cytology constitute one input into the holistic information perspective needed to formulate an acceptable or phylogenetically-reliable system of grouping diverse algae.

### RANGE OF THALLUS ORGANIZATION

The algae exhibit an enormous diversity of thallus organization, ranging from the microscopic unicellular habit at one extreme to the giant macroscopic seaweeds such as *Macrocystis* which may be over 100 metres long.

Unicellular forms are widespread among several algal phyla except the Phaeophyta. They may be motile or non-motile. Most motile algae move by means of flagella, e.g., *Chromulina*, *Euglena*, and *Cryptomonas*, but no such flagellate stages are met with in the Cyanophyta and Rhodophyta. Some unicellular algae move by putting out fine protoplasmic projections, e.g., *Chrysamoeba* and *Dinamoebidium*. Non-motile coccoid algae show a wide range of cell shapes, as in the orders Chlorococcales and Heterocapsales.

Unicellular algae may be uninucleate (e.g., *Chlamydomonas* and *Cosmarium*) or multinucleate (e.g., *Chlorococcum*).

Multicellular algae may be colonial (e.g., *Gonium*, *Pediastrum*, *Dictyosphaerium*, and *Hydrurus*), filamentous (e.g., *Ulothrix*, *Tribonema*, and *Ectocarpus*), or siphonous (e.g., *Botrydium*, *Codium*, *Caulerpa*, and *Vaucheria*). The filamentous forms may be simple and unbranched (e.g., *Microspora*) or branched (e.g., *Stigeoclonium* and *Pylaiella*). Some branched, filamentous algae have their branches disposed in a prostrate system from which arises an erect or projecting system of branches. These algae are described as heterotrichous and are exemplified by *Stigeoclonium* and *Ectocarpus*. In some cases, the filaments and their branches aggregate together in such a manner as to mimic the arrangement of cells in a parenchymatous tissue. These algae are pseudoparenchymatous and often form elaborate macroscopic thalli. Good examples are *Udotea*, *Mesogloea*, *Chordaria*, *Batrachospermum*, *Cumagloia*, and *Nemalion*.

Truly parenchymatous habit is also known. In these forms, cells of the basic filament divide in more than one plane, followed by non-separation of the division products. Examples of truly parenchymatous habit are met with in *Ulva* and *Porphyra*.

Siphonous algae have multinucleate thalli in which the individual cells are



not commonly delimited by cross walls. The simplest examples are *Protosiphon* and *Botrydium*. These are vesicular, unicellular, and multinucleate. In *Vaucheria*, the siphonous filament is branched and multinucleate with septa being formed only in connection with the production of reproductive stages.

Some marine algae belonging to the Chlorophyta, Phaeophyta, and Rhodophyta form fairly large and elaborate bodies with considerable anatomical differentiation into tissues reminiscent of those found in vascular plants. The kelps belonging to the order Laminariales are particularly noteworthy. They form leaf-like and stem-like parts which are internally differentiated into meristoderm (corresponding to epidermis of higher plants), cortex, and medulla. Some of the cells in the medulla become modified as phloem-like structures which even form "sieve-plates". *Undaria*, *Laminaria*, and other such algae also produce mucilaginous canals and mucilage glands.

## CLASSIFICATION

Before the advent of electron microscopy, the chief bases underlying the primary classification of algae into major groups were: (1) the photosynthetic pigments, (2) the chemical nature of cell walls and food reserves, and (3) the form and flagellation of the motile reproductive stages. This kind of approach which relied heavily on physiological and biochemical characteristics of vegetative stages often led to the inclusion of morphologically identical algae differing in pigmentation, etc., in different phyla. Thus, in the widely-used system of classification proposed by Fritsch (1935), such morphologically similar but physiologically dissimilar algae as *Stigeoclonium* and *Ectocarpus* came to be classified in two different classes, the Chlorophyceae and Phaeophyceae.

Fritsch classified the algae into 11 classes, viz., the Chlorophyceae, Xanthophyceae, Chrysophyceae, Bacillariophyceae, Cryptophyceae, Dinophyceae, Euglenineae, Chloromonadineae, Phaeophyceae, Rhodophyceae, and Myxophyceae (Cyanophyceae). Some other classical phycologists, e.g., F. Oltmanns, A. Pascher, G.M. Smith, J.E. Tilden, M.O.P. Iyengar, H. Hirose, and G.F. Papenfuss, also mainly based their proposals for algae classification on the above physiological criteria and divided the algae into 7–13 phyla, the number of classes varying from phycologist to phycologist. Thus, both Papenfuss and Hirose (Hirose, 1959) recognized 13 classes. Nevertheless, the various systems of classification differ widely as regards the status and rank assigned to various taxa, and a given class or phylum of one authority may not be exactly equivalent to a class or phylum of the same name in the sense of another expert.

Following the examination of diverse algal cells under the electron microscope, a host of additional criteria, of a morphological or ultrastructural nature, became available to phycologists and this has greatly stimulated many modern phycologists to propose new systems of classification which take into account the fine structural attributes of chloroplasts, pyrenoids, eyespots, flagella, and other organelles. Another major development during the last three decades has been an appreciation of the two basic kinds of cellular organization, namely, prokaryotic and eukaryotic. This has highlighted the fundamental disparity between the blue-green algae on the one hand and all other algae on the other hand. The preponderance of opinion now is to emphasize this basic structural disparity, and to rename the blue-green algae (Cyanophyceae) as cyanobacteria and to place them under the jurisdiction of the Bacteriological Code rather than the Botanical Code (Stanier and Cohen-Bazire, 1977). However, although this proposal has been



enthusiastically welcomed and adopted by bacteriologists, microbiologists, and many phycologists, it is by no means uncontested (Lewin, 1976a; Golubic, 1979). The prokaryotic blue-greens show striking cytological resemblances with true bacteria but at the same time they are more like the eukaryotic algae and green plants as regards their photosynthetic metabolism. However, these extremely important photosynthetic prokaryotes have since long been studied/taught by phycologists but neglected by bacteriologists. Their descriptions are encountered in books on botany rather than microbiology or bacteriology. Moreover, their pigmentation, oxygenic photosynthesis, and algal habitats are still deemed to be sufficient to warrant their continuation, at least for another few years, in a book on algae.

One consequence of the many studies undertaken on the fine structure of algae has been a proliferation of orders, classes, or even phyla. There is no indication of any consensus emerging as to an acceptable system of classification, and the problem is particularly serious with the "green" algal complex which has been the cause of much debate among phycologists. Hopefully, this problem may be solved when a much larger number of the green algal taxa have been investigated cytologically and ultrastructurally.

Another recent spin-off of cytological and ultrastructural research has been the creation of a new phylum of algae called the Prochlorophyta (Lewin, 1976b). This phylum includes certain unicellular green algae which show prokaryotic organization but lack phycobilin pigments as well as phycobilisomes (Table I). The genus *Prochloron* occurs in association with marine didemnid ascidians and cannot be grown in laboratory culture (Herdman, 1981). *Prochloron* contains a fair-sized genome (around  $3.59 \times 10^9$  daltons) which places it at the upper limit of genome sizes recorded for unicellular prokaryotes. By comparison, the genome size of the unicellular eukaryotic *Chlorella* is around  $6 \times 10^{10}$  daltons, whereas that of the cyanelles or chloroplasts of the anomalous alga *Cyanophora paradoxa* is only around  $1.2 \times 10^8$  daltons. Interestingly, despite repeated attempts, these cyanelles have failed to grow in culture media.

As *Prochloron* is prokaryotic, we may expect that it will soon be claimed by the bacteriologists and its class, the Prochlorophyceae, renamed as Prochlorobacteria.

In view of the prevailing uncertainty and the lack of consensus about the number of classes and phyla of algae (or even about which organisms may or may not be included among the algae), the classification scheme used in this book is arbitrary and tentative and by no means final.

## ALGAL CYTOBIOLOGY AND HUMAN WELFARE

Perhaps the most important biological process in the world is photosynthesis whereby green plants fix carbon dioxide into carbohydrates, using sunlight, thereby converting the light energy into chemical form. Like higher plants, algae carry out photosynthesis when growing in light. Biological nitrogen fixation is a process that may be second only to photosynthesis in its significance to modern agriculture (e.g., see Fig. 1-1) and general human welfare. Most algae lack the capacity to fix nitrogen but many blue-green algae can fix it into useful nitrogenous compounds. Together with nitrogen-fixing eubacteria (such as *Rhizobium* and *Azotobacter*), the blue-greens probably account for about one-half of the total amount of nitrogen fixed annually. Modern agriculture requires enormous inputs of nitrogenous fertilizers and many chemical factories manufacture fertilizers throughout the world; these factories use tremendous amounts of energy and some



current estimates suggest that the total amount of energy required for the worldwide production of ammonium fertilizers may equal or exceed some two million barrels of oil per day (see Shanmugam and Valentine, 1975).

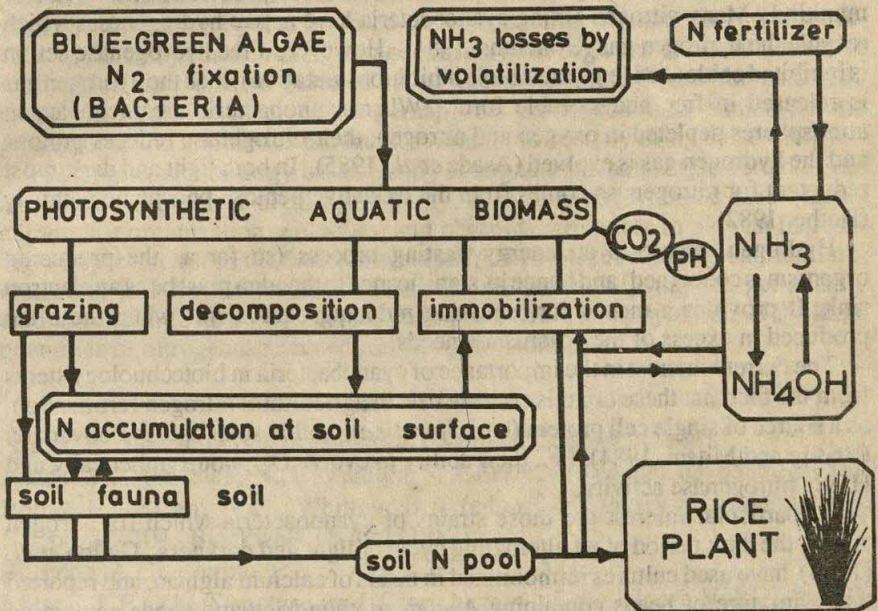
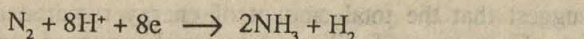


Fig. 1-1 Role of nitrogen-fixing cyanobacteria in a rice field (after Roger, 1986).

In view of the increasing energy demands and rising costs of chemically making nitrogenous fertilizers, much attention is now being given to nitrogen-fixing bacteria and blue-green algae. The blue-greens particularly are of great importance because of their unique combination of an oxygenic photosynthesis and the fundamentally anaerobic process of nitrogen fixation. At the cellular level, these organisms, when growing under aerobic conditions, compartmentalize their oxygenic photosynthesis and the oxygen-sensitive nitrogenase in two distinct cell types, viz., the vegetative cell and the heterocyst. But when they grow under anaerobic or microaerobic conditions, they carry out both the nitrogenase-mediated reactions and the bacterial type of anoxygenic photosynthesis in their vegetative cells. The key enzyme involved in the conversion of nitrogen gas into ammonia, viz., nitrogenase, is exceptional among enzymes in consuming as much as 15 moles of ATP per mole of nitrogenous substrate reduced to  $\text{NH}_4^+$  level. Furthermore, this enzyme is highly sensitive to oxygen and is cold labile. And yet, despite such relatively stringent requirements for biological nitrogen fixation, a large number of blue-green algae and symbiotic as well as free-living eubacteria possess the basic biochemical and cellular machinery needed to support nitrogen fixation.

The enzyme nitrogenase which catalyzes the conversion of  $\text{N}_2$  into  $\text{NH}_3$  consists of two components, an iron-containing protein and a molybdenum-plus-iron-containing protein. When supplied with Mg, ATP, and electrons from a low potential electron donor, the two components together catalyze the reaction:





The evolution of hydrogen appears to be an obligatory part of the reaction mechanism, and offers a great potential as a valuable process for the future of mankind. Many nitrogen-fixing cyanobacteria have active hydrogenases which recycle the hydrogen gas (Houchins, 1984). However, if the hydrogenase action is inhibited or blocked (e.g., metabolic inhibition, mutation), then the hydrogen gas is released in free and storable form. When cyanobacteria are incubated in atmospheres depleted in oxygen and nitrogen, their nitrogenase reduces protons and the hydrogen gas is evolved (Asada *et al.*, 1985). In both light and dark, most reductant for nitrogenase comes from the oxidative pentose phosphate pathway (Bothe, 1982).

Hydrogen release is an energy-wasting process (so far as the producing organism is concerned) and hence its significance to the alga must be as an electron sink. It provides a means for removing reducing equivalents when these are produced in excess of the organism's needs.

The current interest in the importance of cyanobacteria in biotechnology stems from the fact that these organisms have role in agricultural nitrogen fertilization, as a source of single cell protein (e.g., *Spirulina*), and in solar energy conversion (Reddy and Mitsui, 1984), i.e., their ability to evolve  $\text{O}_2$  photosynthetically and  $\text{H}_2$  via nitrogenase activity.

Of particular interest are those strains of cyanobacteria which fix nitrogen during the dark period of an alternating cycle of light and darkness. Gallon *et al.* (1984) have used cultures immobilized in beads of calcium alginate and reported that a mixture of beads containing *Anabaena cylindrica* and beads containing *Gloeotheca* sp. (a non-heterocystous alga which can fix nitrogen aerobically) show a significant rate of  $\text{N}_2$  fixation throughout an alternating cycle of 12 hr light and 12 hr darkness. Gallon *et al.* believe that in such a system, *Anabaena* may be fixing nitrogen in the light and *Gloeotheca* does so in the dark. The use of such mixed cultures is obviously of great value in any industrial process that employs cyanobacteria for  $\text{H}_2$  evolution using natural daylight which, though free, alternates with the dark periods (night). Such a system would continue to generate  $\text{H}_2$  during the darkness; indeed, Asada and Kawamura (1984) have demonstrated hydrogenase activity in the non-heterocystous, non-nitrogen-fixing cyanobacterium *Microcystis* which evolves hydrogen rapidly upon anaerobic incubation in the dark. Asada *et al.* (1985) have also shown that in heterocystous cyanobacteria (Fig. 1-1), the nitrogenase activity is maximal in the light but that they also often show some activity in the dark. Cyanobacteria such as *Gloeotheca* and *Microcystis* are important in that they evolve  $\text{O}_2$  during the day, and  $\text{H}_2$  during the night, thus eliminating the need to separate the two gases as would be the case were the two gases produced simultaneously (Gallon, 1985).

Hall *et al.* (1985) have immobilized cultures of heterocystous cyanobacteria and used them successfully for production of  $\text{NH}_4^+$ .

In recent years, protoplast fusion techniques have offered much promise of tapping sources of genetic variation hitherto unavailable because of sterility and compatibility barriers between species and genera. Burgoon and Botino (1976) have shown that isolated plant cell protoplasts can incorporate within them certain nitrogen-fixing blue-green algae. Serious attempts are underway in many laboratories to transfer the genes for nitrogen fixation from nitrogen-fixing bacteria or blue-green algae into nonlegumes. Recombinant DNA techniques are being increasingly pressed into service to move *nif*-genes into suitable host cells with a



view to increasing the spectrum of biological systems that can fix nitrogen.

Genetic manipulation of photosynthetic prokaryotes is an area of cytobiology that is currently generating much excitement. Much research is being done on plasmids or extrachromosomal DNA. It may be possible to tailor some suitable photosynthetic prokaryotes for large-scale production of industrially and medically useful compounds. An advantage of using these organisms is the relative ease with which they can be cultured rather inexpensively. Genes for antibiotic production, for instance, could perhaps be introduced into photosynthetic prokaryotes and antibiotics produced at minimum cost, by using solar energy.

Plant molecular biology has currently emerged as an amazingly verdant field and certain aspects of algal cytobiology, such as photosynthetic membranes, chloroplast organization, *nif*-genes, and plasmids, are likely to play an increasingly important role in the future progress of plant molecular biology, agriculture, medicine, and human welfare. Plasmids, in particular, may be genetically engineered (Lau *et al.*, 1980) to play an important role in the synthesis of the large quantities of nitrogenase needed for nitrogen fixation.

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## 2 Cell Structure—Prokaryotic

### INTRODUCTION

The two basic types of cell met with in living organisms are prokaryotic and eukaryotic. The bacteria, blue-green algae, and prochlorophytes are prokaryotic, whereas the remaining algal classes, fungi, bryophytes, other plants and animals are all eukaryotic. Prokaryotic organisms lack definite, organized nuclei, mitochondria, and (in plants) chloroplasts. Eukaryotic organisms have firm, complex chromosomes made up of nucleic acids associated with histones or other proteins. The eukaryotic cells contain at least two distinct types of genome—one of the nucleus and the other of the mitochondrion. Photosynthetic eukaryotes also have a third—that of the plastid. Prokaryotes have a much simpler chromosome, devoid of histones or other proteins. The cell walls of prokaryotes contain certain characteristic mucopeptides and acetylmuramic acid but the eukaryotic cell walls lack these components.

Fox *et al.* (1980) divide the living organisms into two kingdoms, Prokaryota and Eukaryota. The prokaryotes are in turn classified into two major subgroups: (1) the archaeobacteria and (2) the eubacteria. Cyanobacteria are included in the group eubacteria. Very soon we may expect the Prochlorophyceae also to be included in the eubacteria.

Table I summarizes the main distinctions between prokaryotes and eukaryotes.

This chapter is mainly devoted to the cyanophytes as much more is known about them than about *Prochloron* (Lewin, 1976) and *Prochlorothrix*. This class Prochlorophyceae includes photosynthetic prokaryotic organisms that contain both chlorophylls a and b but lack phycobilins. The cell wall and cell structure broadly resemble those of cyanobacteria and there are no membrane-bound subcellular organelles. However, the thylakoids occur in pairs or stacks as in the eukaryotic Chlorophyceae (Withers *et al.*, 1978).

In cyanobacterial cells, the photosynthetic, respiratory, and genetic apparatuses are present but are not delimited from each other by any bounding membrane of their own. No sharp boundaries divide the cell into special regions. Rather, there are several cell components with characteristic fine structure. These are distributed (Figs. 2-1, 2-2) through the cell in patterns varying from species to species and also in different developmental stages in the same species.

These cells have an elaborate photosynthetic membrane system (Figs. 2-3, 2-4) composed of single thylakoids and a central nucleoplasmic area which is usually fibrillar or granular or both. Other characteristic attributes of the cell include various kinds of granular inclusions, a rigid, several-layered cell wall, and a fibrous sheath over the cell wall. In fact, the whole cell constitutes a single physiological and biochemical unit. The characteristic collective properties of cyanobacteria include oxygenic photosynthesis, chromatic adaptation, nitrogen fixation, and a capacity for cellular differentiation by the formation of heterocysts, akinetes, baeocytes, and hormogonia.



**Table I** Some properties of prokaryotes and eukaryotes (after Schiff, 1981)

Characteristic	Prokaryotic cells	Eukaryotic cells
Size	Up to 10 $\mu\text{m}$	Up to 100 $\mu\text{m}$
Membrane-bound organelles	Absent	Present
Cell wall	Generally present	Present or absent
Endoplasmic reticulum	Absent	Present
Golgi bodies	Absent	Present
Ribosomes	70S	80S
tRNAs	Complete set	Unique complete set
Chloramphenicol inhibits protein synthesis	Yes	No
Cycloheximide inhibits protein synthesis	No	Yes
DNA genome	Usually single, circular DNA molecule	Many DNA molecules associated with histones in definite chromosomes
Spindle or mitotic figures	No	Present
Location of phosphorylative cell respiration	Cell membrane	Mitochondria
Location of photosynthesis	Cell membranes or thylakoids	Chloroplasts
Reproduction	Fission, fragmentation, parasexual recombination	Mitosis, meiosis, true sexuality

### SHEATHS

The cells, filaments, or colonies of most cyanobacteria are covered with extracellular investments in the form of distinct sheaths, slimy shrouds, or cloud-like slimy sheaths. Well-defined, distinct sheaths occur in many terrestrial species of Scytonemataceae, Stigonemataceae, *Lyngbya*, *Porphyrosiphon*, etc. Cloud-like slimy investments are characteristic of aquatic species of *Nostoc*, planktonic and amphibious *Anabaena*, and other aquatic forms such as *Microcystis*. Many species of *Nostoc* form slimy sheaths, and in these species motility is inversely related to the amount of slime present (Martin and Wyatt, 1974). According to Martin and Wyatt, investments seem to characterize ecological types rather than following taxonomic lines.

The extent, consistency, colour, and texture of the mucilage sheath varies from species to species, in response to habitat conditions. The sheath stains easily with alcian blue or ruthenium red, and can be rendered conspicuous by mounting the alga in a drop of diluted India ink. Chemically, it is made up of



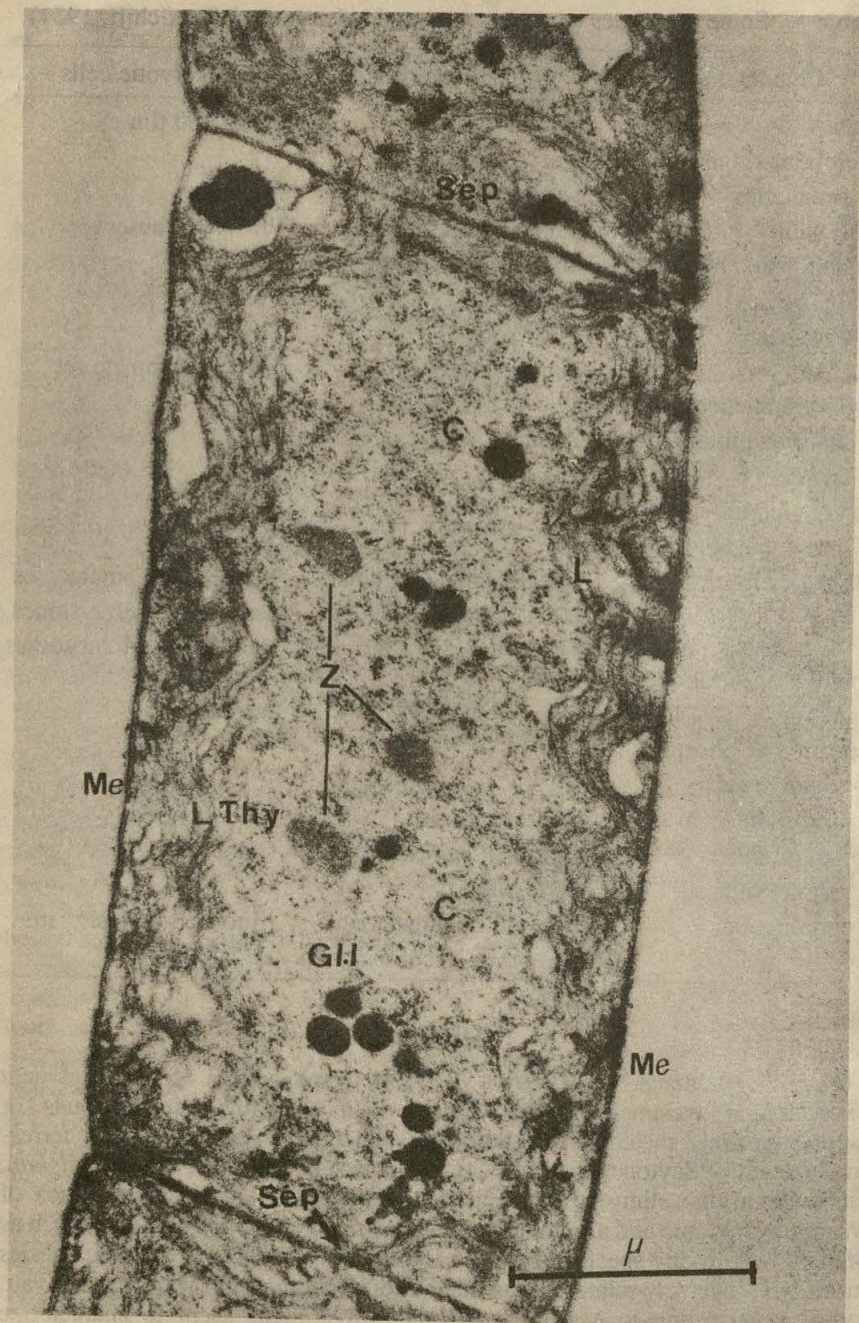


Fig. 2-1 Electron micrograph of section through *Oscillatoria brevis*, giving an overview of the general organization of cyanobacterial cell. Me, cell wall and membrane; Thy, thylakoid; G.I., lipid globules; Sep, septum. (x32,250.) Courtesy M. Lefort-Tran.



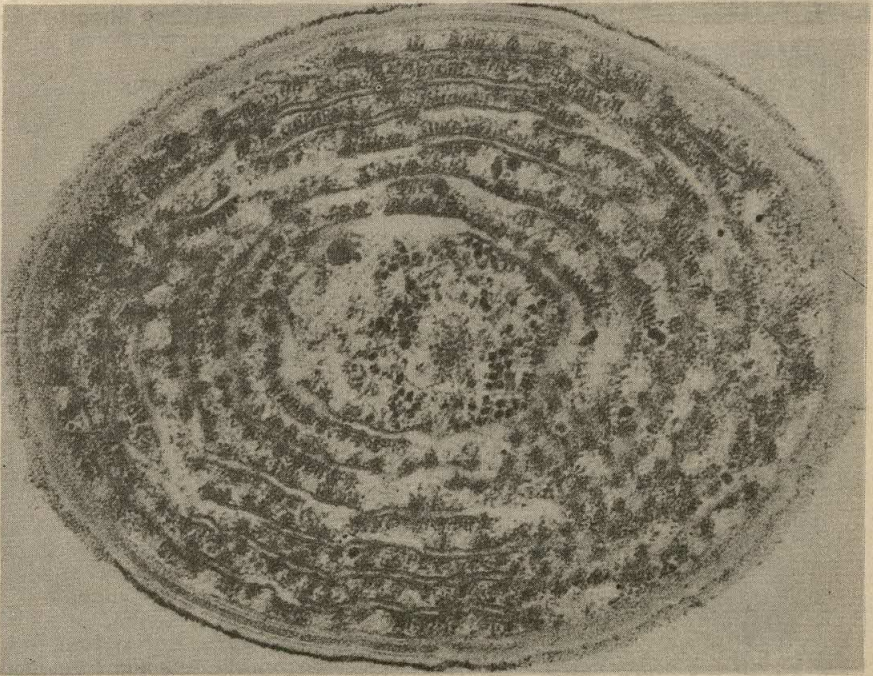


Fig. 2-2 Electron micrograph of section through a cell of *Synechococcus lividus*, showing the typical prokaryotic ultrastructure. (x50,750.) Courtesy E. Gantt and M.R. Edwards.

acid mucopolysaccharides and pectic acids. It is commonly believed that mucilaginous sheaths serve to protect the cells against atmospheric oxygen and create locally anaerobic conditions in which the protoplasm of these organisms thrives best.

The sheaths exhibit a characteristic fibrillar ultrastructure with the fibrils being disposed reticulately within an amorphous matrix. The sheaths of filamentous species are usually tubular with open ends.

A recently-discovered marine Chroococcalean blue-green alga, named DC-2, is exceptional in forming a characteristic outer cell wall sheath, composed of a series of parallel ridges spaced about 300 Å apart. These ridges are serrate in cross section, branched at ends of the cells, and run parallel to its long axis (Kursar *et al.*, 1981).

### CELL WALL

The cell wall, which lies internal to the sheath, is typically four-layered. The four layers, LI-IV, lie outside the plasmalemma in most blue-greens. In addition to these four, *Microcystis* and *Gomphosphaeria* have a faint proteinaceous layer external to the L-IV layer (Cmiech, 1981, cited by Reynolds *et al.*, 1981).

The L-IV layer has a globular substructure which contrasts with the fibrous or non-globular nature of the innermost layer of the sheath (Lang, 1968).

The cell walls of *Synechocystis*, *Anacystis*, and some other cyanobacteria contain some myxoxanthophyll, β-carotene, echinenone, zeaxanthin, and some



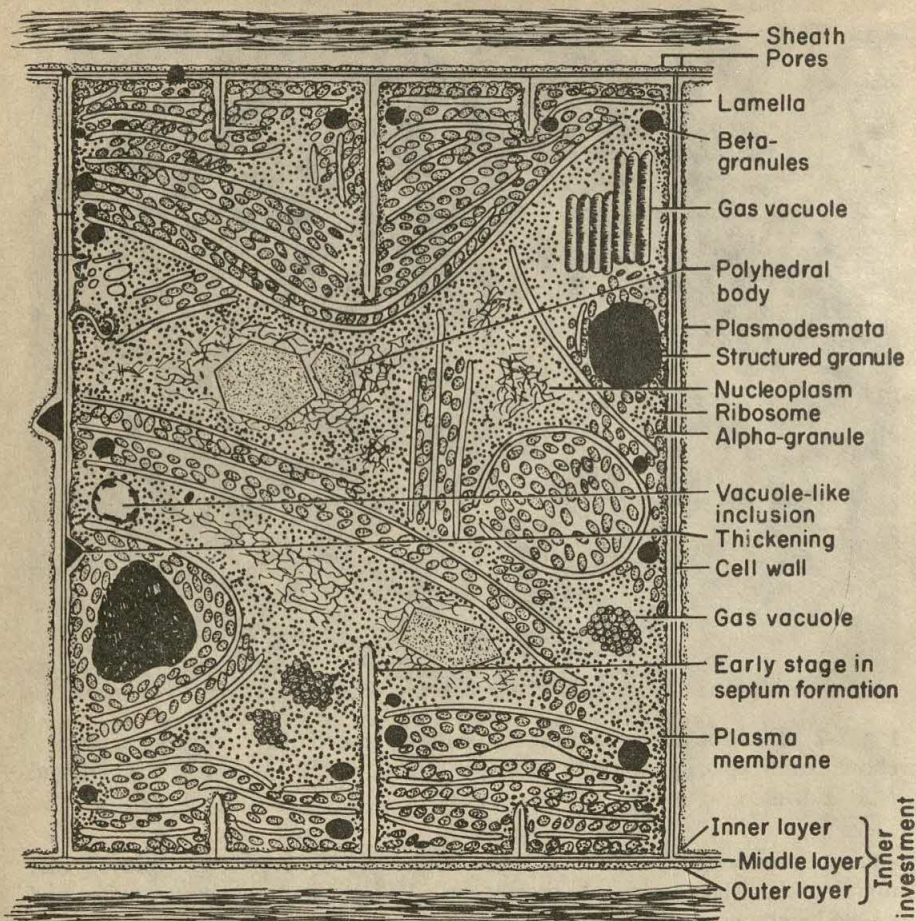


Fig. 2-3 Diagrammatic sketch of a typical cyanobacterial vegetative cell to illustrate its fine structure.

other carotenoids (Jürgens *et al.*, 1985). Some other constituents of these cell walls include proteins, polysaccharides, lipopolysaccharides, lipids, galactolipids and, of course, peptidoglycans. Cell envelopes of *Aphanothece halophytica* and *Oscillatoria limnetica* contain some glycoprotein which might be involved in gliding.

Proteins are regular constituents of cyanobacterial cell envelopes. They occur in outer membranes. Some proteins are associated with peptidoglycan (Jürgens *et al.*, 1985). Others are involved in pore formation (Flügge and Benz, 1984). Proteins also contribute to cell surface hydrophobicity in *Phormidium* sp. (Bar-Or *et al.*, 1985). The hydrophobic proteins make it possible for the cell to adhere to the benthos. Another example of cell surface modulation is the change of hydrophobicity to hydrophilicity during the formation of hormogonia in filamentous, benthic cyanobacteria (Fattom and Shilo, 1984).

In addition to proteins, the outer membranes of cyanobacteria contain lipid and lipopolysaccharide, thus suggesting a Gram-negative type of cell wall. This is surprising in view of the fact that cyanobacteria give a positive reaction



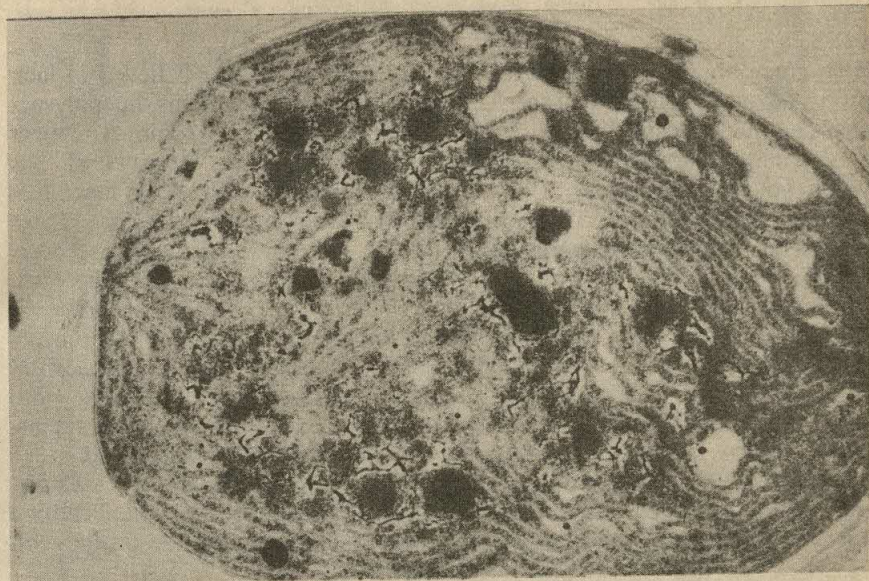


Fig. 2-4 Electron micrograph of section through a cell of *Pleurocapsa*, showing DNA fibrils, thylakoids, and polyhedral bodies. (x17,200.) Courtesy K. Ueda.

with the Gram stain (Jürgens and Weckesser, 1985). The reported occurrence of polysaccharide covalently bound to peptidoglycan, in a *Synechocystis* points to strong affinity with Gram-positive organisms, as this condition has not been reported for any Gram-negative bacteria.

The cell wall of *Oscillatoria rubescens* is a four-layered structure. Each of these layers has a globular substructure and is about 10 nm thick.

The cell wall of blue-green algae contains mucopolymers, generally composed of muramic acid, glucosamine, diaminopimelic acid, glutamic acid, and alanine. Of these five, diaminopimelic acid is restricted only to prokaryotes.

The L-II layer is largely or entirely peptidoglycan. The L-IV contains both lipopolysaccharides and proteins (Stanier and Cohen-Bazire, 1977).

In the *Pleurocapsales*, the cell wall has an additional, fibrous layer (called F-layer) external to the L-IV. This additional layer resembles in fine structure the tubular sheaths of the *Oscillatoriaceae* but is very closely appressed to the outer surface of the L-IV (Waterbury and Stanier, 1978). During cell growth, the F-layer continues to enlarge and it also plays an important role during binary fission and during the formation of reproductive cells, called baeocytes, which arise by multiple fission of vegetative cells.

Guglielmi and Cohen-Bazire (1982) studied the peptidoglycan wall layer of several cyanobacteria and observed the presence of pores in it. These pores have a rather electron-translucent peripheral zone and an electron-opaque central core. The core in turn has some fine perforations that form a circle around a central microperforation. In some cyanobacteria, e.g., *Oscillatoria* spp., only one ring of pores occurs in close proximity to the cross wall. In *Spirulina* spp., the pores are distributed along partial rings in the concave region of the peptidoglycan. In *Pseudanabaena*, the pores tend to occur either within a circular zone or in several concentric rings.



## PILI

Blue-green algae lack flagella and until recently were also believed to lack pili. The outer membrane of these organisms consists of proteins, lipopolysaccharides, and phospholipids. Many cyanobacteria are now known to bear certain surface structures outside their outer cell membrane. Vaara (1982) has worked on several species of Chroococcacean cyanobacteria and has found that their surface structures are broadly similar to those of eubacteria. These Chroococcacean cyanobacteria show piliation: *Microcystis firma*, *Synechocystis* (several species), some strains of *Synechococcus*. All gliding strains form pili. However, Stewart (1980) reports that pili may not be essential for movement since some motile strains lack pili. Pili play an important role in prokaryotic/eukaryotic cell recognition phenomena (Jones, 1977). Cyanobacterial pili (Figs. 2-5, 2-6, 2-7) are long, stiff threads of even width and seem to possess a subunit structure, the subunits being made of pilin. In some strains, e.g., *Synechocystis*, the pili form bundles resembling those of *Neisseria*.

Perkins *et al.* (1981) have reported the presence of straight-walled, cylindrical spinae in some planktonic strains of *Synechococcus*. An unidentified marine coccoid cyanobacterium has several spinae scattered on the cell

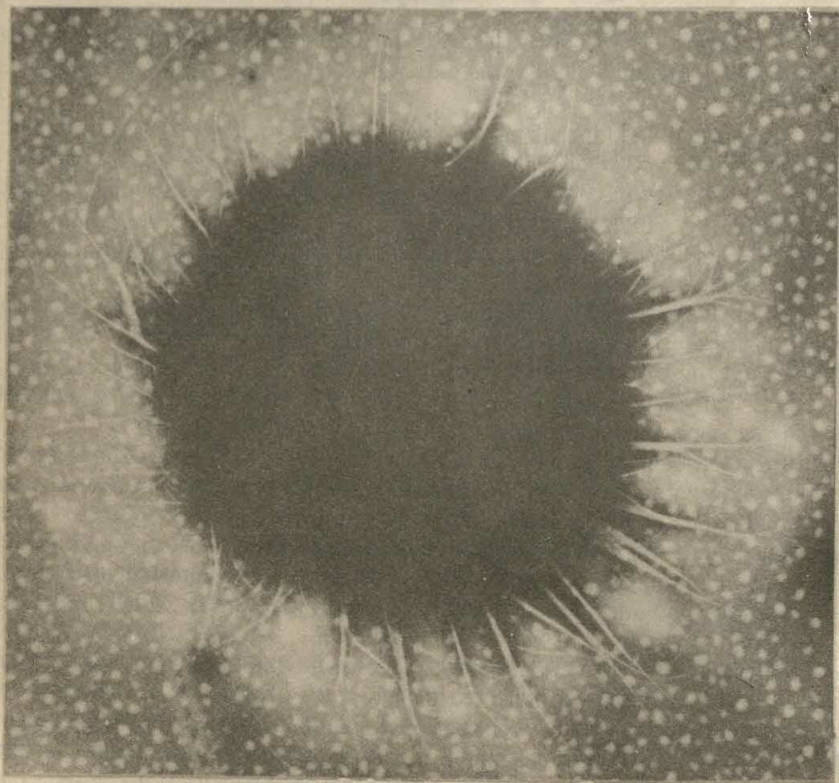


Fig. 2-5 Negative staining of *Synechocystis* CB3 cell stained with 2% phosphotungstic acid in 0.1 M sodium phosphate buffer, pH 6.5. The pili attach together and form bundles around the cell. (x20,000.)  
Courtesy T. Vaara.



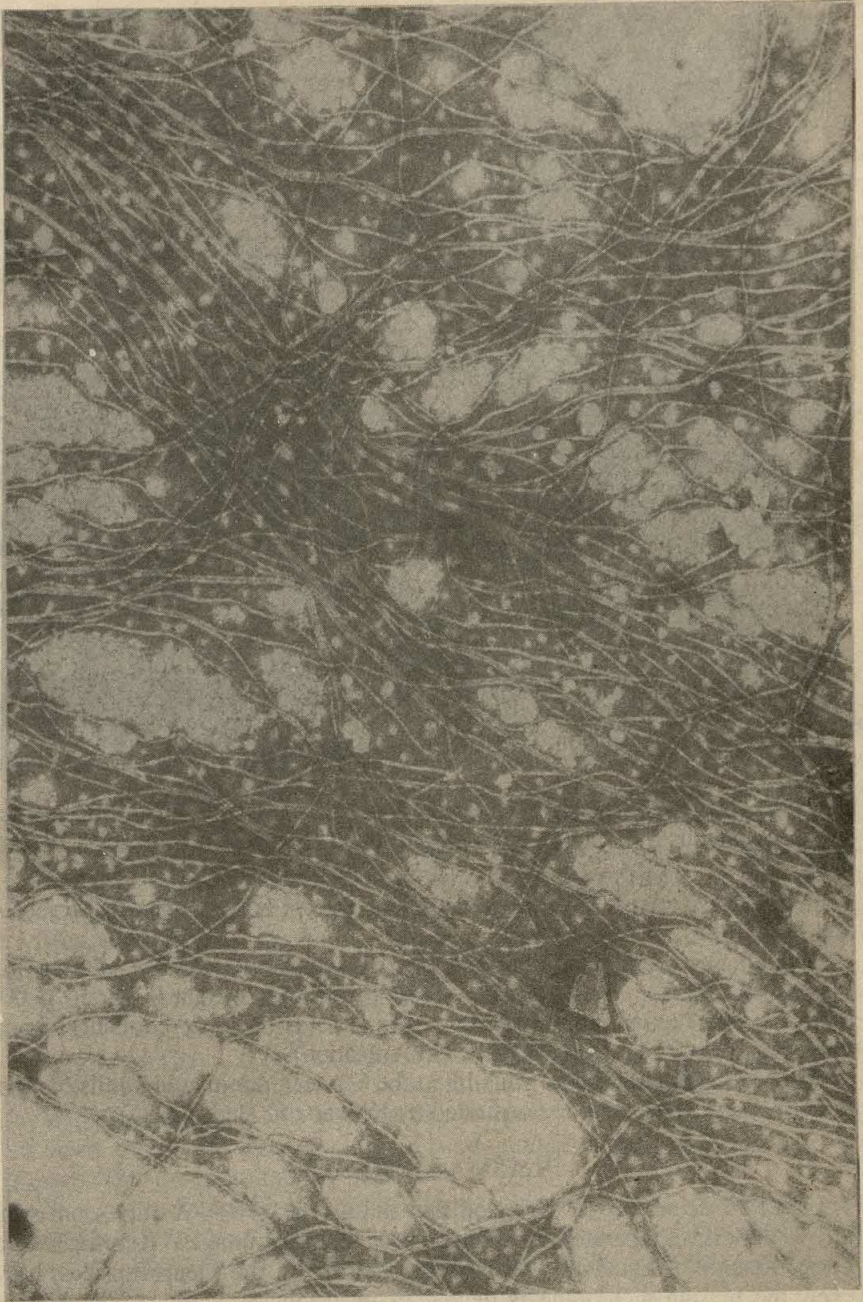


Fig. 2-6 Negative staining of isolated pili of *Synechocystis* CB3, stained with 1% aqueous phosphotungstic acid, pH 6.5. (x85,000.)  
Courtesy T. Vaara.



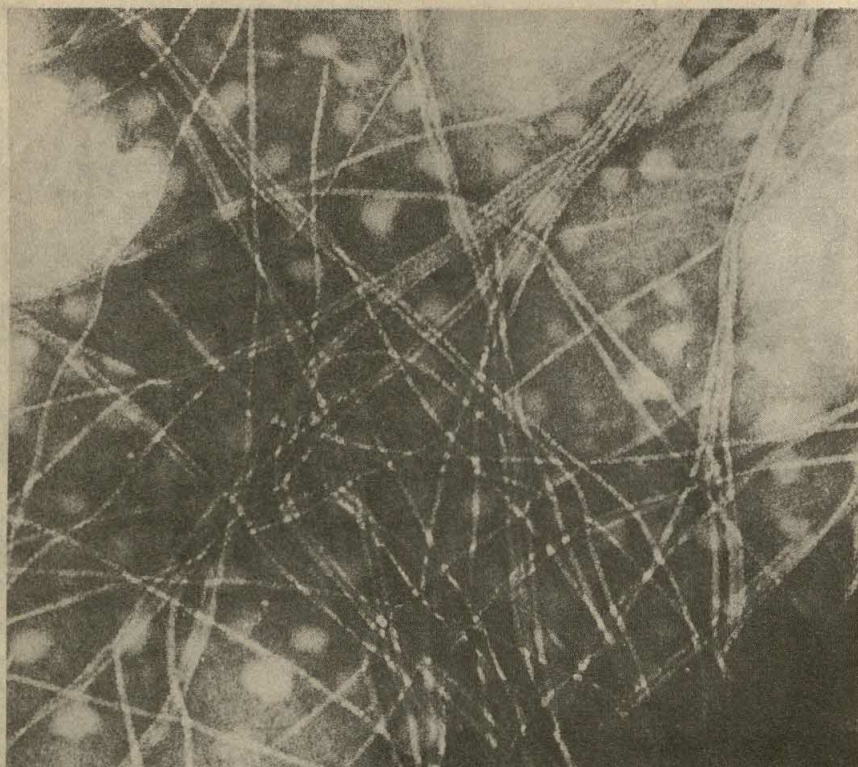


Fig. 2-7 Negative staining of isolated pili of *Synechococcus* PCC 7502, stained with 1% aqueous phosphotungstic acid, pH 6.5. ( $\times 100,500$ .) Courtesy T. Vaara.

surface. These spinae are conical and tapered from base to apex (Easterbrook and Rao, 1984).

Vaara has recently isolated the pili of *Synechocystis* CB3. These pili (Fig. 2-6) contain pilin subunits whose molecular weight is about 21,000 daltons. The subunits not only have amino acids but also contain glucosamine residues (T. Vaara, personal communication).

The rare formation of a pilus-like tube between conjugating cells of *Anacystis nidulans* has been demonstrated by Kumar and Ueda (1984).

### PLASMA MEMBRANE

This covers the surface of the cytoplasm and is typically 80 Å thick, consisting of two dark lines separated by a light area in sectional images. It forms several projections or invaginations into the cytoplasm. In the Pleurocapsales, however, the plasma membrane is devoid of intrusions (Waterbury and Stanier, 1978). Being a unit membrane of the type found in many biological membrane systems, it consists of a lipid bimolecular layer containing abundant proteins (Fig. 2-8). The density of protein particles in the plasma membrane of *Oscillatoria rubescens* is  $2900/\mu^2$  (Jost, 1965).

The plasma membrane is semi-permeable. Jost (1965) assumed that the protein particles are enzymic proteins involved in respiration or in cell wall synthesis.



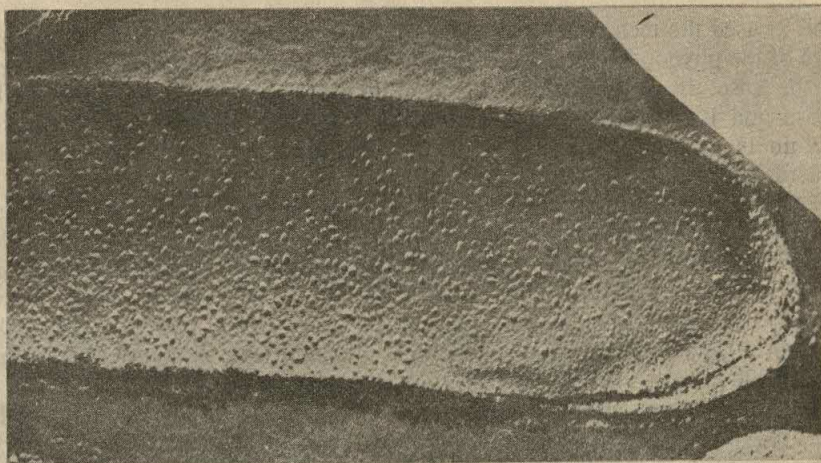


Fig. 2-8 Freeze fracture electron micrograph of *Anacystis nidulans*, showing protein particles on the EF face of the plasma membrane. (x65,000.) Courtesy K. Ueda.

### PHOTOSYNTHETIC MEMBRANES

Flattened vesicles which have photosynthetic activity are called thylakoids. Generally, small cylindrical cells, such as those of *Oscillatoria minima* (Ueda, 1971a) and *Anacystis nidulans*, contain concentrically arranged thylakoids which lie parallel to the cell axis. However, thylakoids are randomly scattered in spherical or ellipsoidal cells. The chromatoplasm and centropylasm can be clearly detectable in the former type of cells, whereas they are sometime difficult to distinguish from each other in the latter cells. Each thylakoid is around 20 nm thick. It consists of two closely appressed unit membranes separated from one another by a space of 3–6 nm.

Thylakoids may be cut by cell wall ingrowth, but they expand their surface area as the cell grows. The number and appearance of thylakoids in cells can change in response to culture conditions. Findley *et al.* (1970) cultured *Chlorogloea fritschii* under two different light intensities, viz., low (20–60 foot candles) and high (700 ft. c). Four to six thylakoids tended to group and lie parallel with each other in cells cultured under the low light condition. Each thylakoid was large in size and had narrow lumen. In contrast, thylakoids did not form groups and were small in size and number in those cells that had been grown under the high light intensity.

Changes in appearance of thylakoids accompanying cell ageing have been reported. Pankratz and Bowen (1963) reported that thylakoids which were separately distributed in young cells, stacked on top of each other in old cells of *Symploca muscorum*. Ueda (1971b) described the form change of thylakoids in *Oscillatoria bornetii*; the thylakoid lumen became swollen, changing the shape from flat vesicles to rounded vesicles after prolonged culture.

The outer surfaces of thylakoids are occupied partly by regular arrays or contiguous rows of spherical or discoidal phycobilisomes. These contain phycocyanin and small amounts of phycoerythrin. Phycobilisomes also occur in the eukaryotic Rhodophyceae and Cryptophyceae. Ganitt and Lipschultz



(1977) used the technique of immuno-electron microscopy to probe the structure of the phycobilisome in the eukaryotic red alga *Porphyridium purpureum*. In this case, the phycoerythrin is located on the outer surface of the phycobilisome and it surrounds phycocyanin and allophycocyanin. The allophycocyanin is located near the base of the phycobilisome, contiguous with the plastid lamellae.

Allophycocyanin is a highly effective light-harvesting pigment which also serves as a conduit for the radiationless transfer of light energy absorbed by other phycobiliproteins to reaction centre chlorophyll. Cohen-Bazire *et al.* (1977) purified allophycocyanins from diverse cyanobacteria. These native proteins are trimeric molecules with the structure  $(\alpha\beta)_3$ . They found that the renatured alpha- and beta-subunits of allophycocyanin were immunologically distinct from each other, and both cross-reacted with the antiserum to the native protein. Trimeric allophycocyanin could be readily reconstituted from the purified alpha- and beta-subunits, and hybrid allophycocyanins were formed. This work of Cohen-Bazire *et al.* has reinforced the view that allophycocyanins are a highly conserved class of proteins.

The phycobilisome is a large multiprotein particle of complex structure. It contains mostly biliproteins which absorb light in the wavelength range of 450–650 nm. This absorbed light is then transferred to the protein-chlorophyll complexes of Photosystems II and I in the photosynthetic lamellae (Glazer, 1982).

The size of most phycobilisomes ranges from  $5\text{--}20 \times 10^6$  daltons. About 85% of a phycobilisome is made up of biliproteins and the remaining part is made of certain linker polypeptides.

Gantt (1980) has classified the phycobilisomes into three types, viz., (1) hemi-ellipsoidal phycobilisomes, found in the Rhodophyta, (2) hemi-discoidal ones, found in the Rhodophyta, cyanobacteria, and certain cyanelles, and (3) the "rod bundle" phycobilisomes, characteristic of the cyanobacterium *Gloeobacter violaceus* (which lacks thylakoids). The type (1) phycobilisomes are larger in size (about  $20 \times 10^6$  daltons) as compared to the other types which are usually  $5\text{--}8 \times 10^6$  daltons.

The type (2) phycobilisome has been studied extensively under the electron microscope. It commonly has a core of three contiguous, disc-like structures, arrayed in an equilateral triangle, from which radiate out up to six rods made up of stacked discs. Phycocyanin, phycoerythrin, and phycoerythrocyanin are present within the rods of the phycobilisomes whereas allophycocyanin is contained in their cores.

The cyanobacterial thylakoid membranes are fundamentally made up of a lipid bimolecular layer in which proteins are inserted. Among the interspersed proteins, there are cytochromes and plastocyanin. Hoshina *et al.* (1984) have shown that a specific form of chlorophyll a is associated with lipids in the thylakoid membranes in *Anacystis nidulans* and that this form often produces temperature-induced absorbance and fluorescence changes in this organism.

The thylakoids are not only the sites for photosynthesis but also for respiration (Whitton and Carr, 1982). In those cells which lack thylakoids (e.g., *Gloeobacter violaceus*—see Rippka *et al.*, 1974), photosynthesis and respiration may both be associated with the plasma membrane (Guglielmi *et al.*, 1981). Possibly the plasma membrane may take over these roles in some other cyanobacteria also (Peschek and Schmetterer, 1980).



Proteinaceous granules of the type now known as phycobilisomes were detected along the photosynthetic thylakoids of the endosymbionts of *Glaucozystis nostochinearum* and *Cyanophora paradoxa* (Figs. 2-9, 2-10) and in the free-living cyanobacterium *Gloeocapsa alpicola* (Fig. 2-11; see Lefort, 1965; Lefort-Tran *et al.*, 1973).

Recent ultrastructural studies of the three-dimensional organization of cyanobacteria, by the application of high-voltage electron microscopy (Nierzwicki-Bauer *et al.*, 1983) have revealed that in *Agmenellum quadruplicatum* the photosynthetic thylakoid membranes entirely surround the central portion of the cytoplasm, thereby demarcating or compartmentalizing it quite



Fig. 2-9 Electron micrograph of section through a cyanelle of *Glaucozystis nostochinearum*. Cya, cyanelle; Nu, nucleoplasm; Chr, chromatoplasm; Fi, DNA fibrils; R, ribosomes; Phy, phycobilisomes. (x23,500.) Courtesy M. Lefort-Tran.





Fig. 2-10 Freeze fracture electron micrograph through cell of *G. nostochinearum*. Isolated, rounded thylakoids show parallel rows of particles on EF fracture face and grooves on PF fracture face, corresponding to phycobilisomes on outer side of photosynthetic membrane. phy, phycobilisomes; thy, thylakoids. (x52,500.) Courtesy M. Lefort-Tran.

distinctly from the rest of the cell. The complete thylakoid system of this organism includes 4–6 pairs of membrane sheets that traverse the entire long axis of the cell. The specialized inclusion bodies consistently occupy specific locations within the cell; thus, both polyphosphate bodies and carboxysomes are always confined to the central cytoplasmic region, sometime in contact with each other. The central cytoplasmic region in this species appears to be a virtually independent compartment bounded by the innermost pair of thylakoid membranes. This may be significant in the context of evolution of the eukaryotic cell. Further, the applications of high-voltage microscopy and other modern techniques for studying three-dimensional structure have shown that, contrary to some published reports (e.g., Echlin, 1964; Holt and Edwards, 1972; Lang and Whitton, 1973) which described the cyanophycean thylakoids as a group of independent discs or flattened sacs, at least in *A. quadruplicatum*



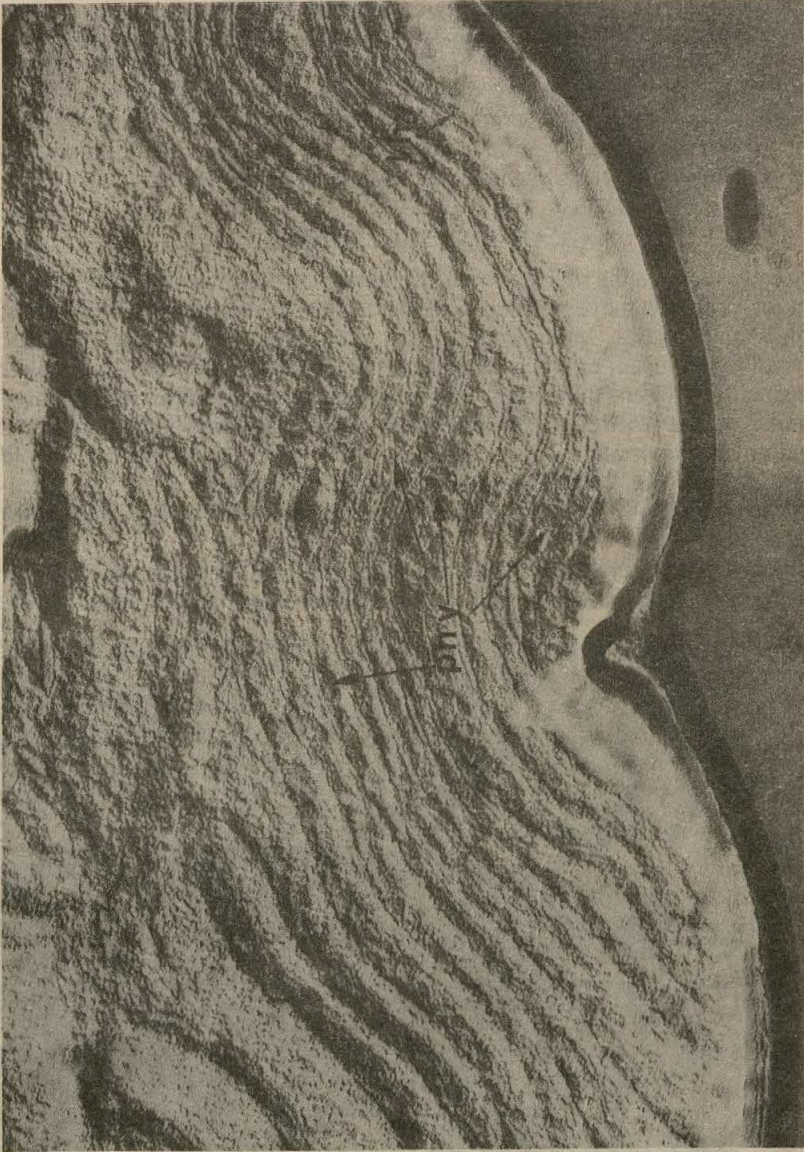


Fig. 2-11 Freeze-etched electron micrograph of section through *Gloeocapsa alpicola*, showing thylakoids. Large phycobilisomes are seen between thylakoids. thy, thylakoids; phy, phycobilisomes. (x93,500.) Courtesy M. Lefort-Tran.



the thylakoids neither form discs nor sacs but constitute an anastomosing network of concentric shells of the type described by Golecki and Drews (1982). According to Nierzwicki-Bauer *et al.* (1983), the thylakoids of this network are in contact with the cytoplasmic membrane at several places, apparently to maintain the overall configuration of the thylakoid system.

Calcium plays some fundamental role in the photosynthesis of *Anacystis nidulans* (Mohanty *et al.*, 1985).  $\text{Ca}^{++}$  is required at a specific site of the electron transport chain near the reaction centre of PS-II. Removal of  $\text{Ca}^{++}$  completely inhibits the PS-II centres.  $\text{Ca}^{++}$  depletion also results in disconnection of the phycobilisomes from the photosynthetic process without preventing energy transfer within the phycobilisome (Mohanty *et al.*, 1985).

### GRANULAR INCLUSIONS

The cytoplasm contains diverse kinds of granules of various sizes and shapes. These include cyanophycin granules, gas vesicles, carboxysomes, ribosomes, polyphosphate bodies, lipid droplets, and poly- $\beta$ -hydroxybutyrate particles. Glycogen (polyglucan) or alpha-granules as well as lipid (osmiophilic) droplets are located between thylakoids. Cyanophycin granules are made of high molecular weight ( $25\text{--}100 \times 10^3$ ) copolymers of only arginine and aspartic acid in equimolar proportion. These granules are especially abundant in old cells and spores (Fig. 2-12). These granules constitute a kind of reserve nitrogen. They stain with neutral red or carmine but not with methylene blue or toluidine blue.

Polyphosphate granules stain reddish blue with toluidine blue due to their metachromasy. They also stain red with methylene blue. These granules were

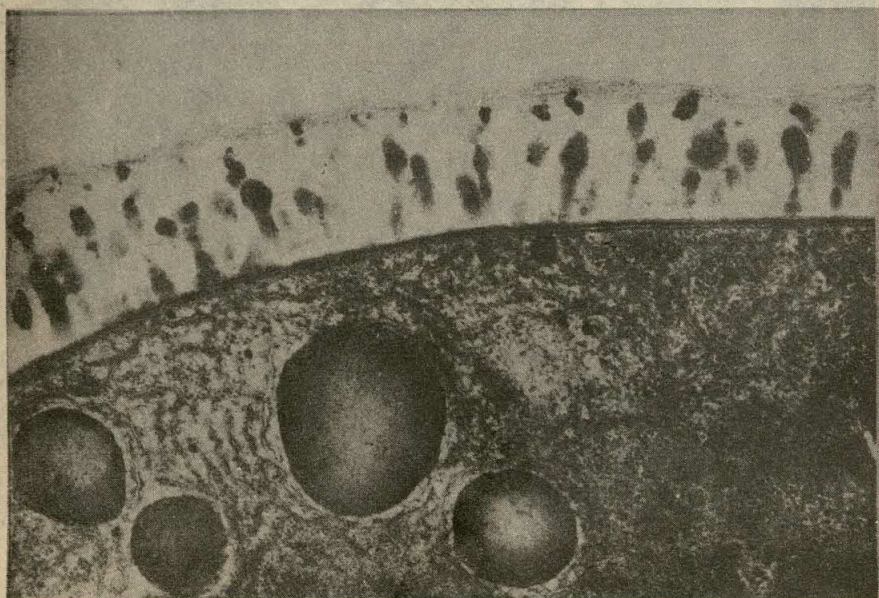


Fig. 2-12 Electron micrograph of part of section through akinete of *Cylandrospermum*, showing thick wall, thylakoids, and cyanophycin granules. ( $\times 23,750$ .) Courtesy K. Ueda.



earlier named as metachromatic or volutin granules. Tischer (1957) observed that cyanophycean cells did not produce these granules when they were cultured in phosphate-free medium. Talpasayi (1963) reported that cyanophycean cells collected from the field had a few small phosphate granules which, however, increased in number and size when the cells were cultured overnight in 0.001 M potassium phosphate under strong illumination. These granules could be solubilized by treating the cells with about 10 per cent trichloroacetic acid solution, and the solute could be precipitated with barium acetate. These observations suggested that the granules contained polyphosphate.

Polyhedral bodies or carboxysomes occur in the central or core region of the cell and contain the important enzyme ribulose biphosphate carboxylase oxygenase (Codd and Stewart, 1976; Shively *et al.*, 1973). Ribosomes are dense granules, 100–150 nm in diameter, which become conspicuous after staining with uranyl acetate. Ribosomes are dispersed throughout the cell. They are of the 70S kind.

Some blue-greens such as *Chlorogloea fritschii* and *Microcystis* contain poly-beta-hydroxybutyrate granules which may be distinguished from the closely resembling polyphosphate granules by the presence of a 3-nm limiting monolayer (Reynolds *et al.*, 1981).

According to Jensen (1984), the inclusions of cyanobacteria may be broadly classified into two groups, those of regular occurrence, and those which occur irregularly. The former category includes thylakoids with associated phycobilisomes, DNA, ribosomes, lipid droplets, glycogen, cyanophycin granules, polyhedral bodies, and polyphosphate bodies. These regular inclusions occur in most or all cyanobacteria. A few species contain, besides the above regular inclusions, the so-called irregular inclusions. The irregular inclusions are of three main kinds, viz., (1) membrane monolayer limited inclusions, e.g., gas vacuoles and poly- $\beta$ -hydroxybutyrate granules, (2) membrane limited inclusions, e.g., prolamellar-like bodies found in some species of *Anabaena* and *Nostoc*, and (3) tubules and filaments, some of which resemble the eukaryotic microtubules. Examples of this last category occur in some strains of *Calothrix*, *Gloeotrichia*, and *Fremeyella*.

### GAS VACUOLES

Many planktonic cyanobacteria characteristically possess gas vacuoles which appear as darkish objects under low power of the microscope and as reddish granules in high power. The most important property of gas vacuoles is that they vanish completely when subjected to pressure. Since they contain certain gases, the presence of gas vacuoles in a cell makes it buoyant, i.e., float on the surface of water. Likewise, disappearance of the vacuoles makes the organism sink down.

The British biologist A.E. Walsby is the leading authority on cyanobacterial gas vacuoles. He has critically studied these structures from diverse angles during the last two decades.

A gas vacuole is made up of a honeycomb-like stacked system of cylindrical, hollow membranes, called gas vesicles, which collapse flat when pressed suddenly. Each gas vesicle cylinder is about 70 nm in diameter, and its length can vary from species to species. Conical caps are present at the ends of the cylinders. The membrane of the gas vesicles is 2–3 nm thick. Walsby



and Buckland (1969) succeeded in isolating highly purified fractions of gas vesicle membranes from *Anabaena flos-aquae* and found that these were colourless, composed largely of protein in which the common amino acids were alanine, valine, isoleucine, leucine, glutamic acid and serine; this protein did not have any sulphur amino acids. Walsby (1971) has shown that, contrary to popular belief, the gas vesicle membranes are freely permeable to such gases as oxygen, argon, nitrogen, carbon dioxide, hydrogen, and methane. In fact, the gas content as well as spectrum of the gas vacuoles are the same as in the surrounding water and the partial pressures of the vacuolar gases are also the same as in the water body (see Fogg *et al.*, 1973).

Three important physiological properties of gas vacuoles are: (1) the vacuole structure cannot be supported by pressure alone, (2) the vacuoles cannot be inflated by gas, and (3) the vacuoles cannot store gas. According to Fogg *et al.* (1973), the gas vesicle is not like a bubble or balloon but rather is a rigid structure from which liquid water tends to be excluded; it may best be compared with a porous pot having a hydrophobic lining through which water cannot diffuse because of unsuitable surface tension but through which gases can diffuse freely. Both Walsby (1969) and Waaland and Branton (1969) postulate a *de novo* synthesis of gas vesicles by the aggregation of their constituent particles in such a manner that with the increase in size, an empty space arises into which gases can diffuse.

Recent researches have conclusively shown that the gas vacuoles do not store nitrogen or other gaseous fermentation products. Their chief functions are related to buoyancy regulation and light shielding. The latter function seems interesting in view of the fact that the vacuole membranes lack any pigments. They seem to shield the cells against unduly high light intensities, possibly through such optical phenomena as refraction, interference, or scattering.

Under the light microscope (phase contrast), a gas vacuole appears as a bright area having irregular outline. Under the electron microscope, however, this vacuole is seen to be composed of numerous gas vesicles. Each gas vesicle is a cylindrical midpiece about 40–100 nm wide, terminating with a conical section at each end. The gas vacuole is a unique kind of organelle, consisting of a thin protein membrane surrounding a space (Staley, 1980). The membrane is quite impermeable to water but is freely permeable to gases. According to Staley, the gas vacuole is not only an organelle for regulating buoyancy but may, in the remote past, have served as an organ of motility. This view is supported by the demonstration of gas vacuoles in archaic, anaerobic organisms such as *Clostridium* and *Methanosarcina*, and in the archaeobacterium *Halobacterium*. There is also some recent evidence to the effect that at least in some bacteria the genes determining gas vesicle synthesis are plasmid-borne.

### UNUSUAL INCLUSIONS

Some cyanobacterial cells have been reported to contain a number of different, unique, intracellular structures such as membranous stacks, spheres, and scrolls in which the membranes are not typical unit membranes. Some spheroidal inclusions occur in intrathylakoidal spaces. In some cells, a variety of filamentous and crystalline arrays of unknown nature occur (Jensen and Bowen, 1970).

Several mesosomes occur in the cells of some cyanobacteria, e.g., *Anacystis*



*nidulans* and *Mastigocladus laminosus* (Balkwill *et al.*, 1984). It seems likely that the mesosomes are sites of ATP formation and for attachment of chromosome.

### MICROTUBULES AND MICROFILAMENTS

These are present not only in eukaryotic cells but also in some prokaryotes. Several species of *Anabaena* have microtubules 10–12 nm in diameter and 50 nm long, occurring in rows. These microtubules project at right angles from one side of an arched plate into the cytoplasm or towards the plasma membrane (Jensen and Ayala, 1976). Or, they are associated with one facet of polyhedral bodies. These organisms also have bundles of microfilaments of varying length, each filament being 2.8 nm in diameter.

*Nostoc muscorum* has striated microtubules (Jensen, 1985). The diameter of these tubules is 18 nm, and their wall has 11–13 units. Their function is unknown.

### THE CELL CORE (NUCLEOPLASM)

The central or core region of the cell is often called nucleoplasm. It contains DNA arranged in the form of a three-dimensional network of about 7-nm thick microfibrils. The DNA is not associated with histones and no firm chromosomes of the type found in eukaryotes are present in cyanobacteria. There are neither mitotic figures nor interphase nuclei with a nuclear membrane.

The DNA base composition in most cyanobacteria examined has been found to range from 35–70 mol % of GC, which is similar to that in diverse eubacteria (Herdman, 1982). However, as a group, the cyanobacteria differ from the eubacteria in exhibiting an extremely wide range of genome size and complexity. Thus, the eubacterial genome size falls within the range  $1 \times 10^9$  daltons to  $3.6 \times 10^9$  daltons (genomes of mycoplasmas are even smaller). In contrast, cyanobacterial genomes range from  $1.6 \times 10^9$  daltons to  $8.6 \times 10^9$  daltons. Some of this DNA may be excess or redundant. The genome of *Anabaena variabilis* has been isolated as a folded, circular chromosome which probably replicates bidirectionally (Herdman, 1982).

The molecular weight of DNA of *Agmenellum quadruplicatum* is about  $3.9 \times 10^9$  daltons and its kinetic complexity ranges from  $2.2 \times 10^9$  daltons to  $2.8 \times 10^9$  daltons (Roberts *et al.*, 1977). Unlike the commonly-studied bacteria, *Agmenellum* may carry two or more chromosomes (i.e., DNA molecules) per cell. Roberts *et al.* have further shown that supercoiled circular molecules constitute up to 5% of the total DNA in this alga.

### PLASMIDS

Plasmids are circular molecules of double-stranded DNA that multiply independently within bacterial host cells and are inherited in a regular manner as those cells multiply. Plasmids account for only some 1–3% of a cell's genome, but this small fraction codes for important accessory genetic traits that are not ordinarily encoded by the bacterial or cyanobacterial chromosome. In bacteria, plasmids are known to be the carriers of resistance to antibiotics and act as vehicles for genetic transfer.

The first indirect indication of the existence of plasmids in a cyanobacterium (*Anacystis nidulans*) was given by Kumar *et al.* (1967). Kumar *et al.*



observed that a strain of *A. nidulans* resistant to high levels of streptomycin, when treated with an acridine dye, lost its resistance or could be "cured", suggesting that streptomycin resistance may be plasmid-borne. Later, Asato and Ginoza (1973) physically observed the presence of a small, circular DNA molecule separate from the main part of the genome in the same species. According to Asato and Ginoza, this circular DNA may not be similar to the resistance transfer factor of bacteria in view of the fact that *A. nidulans* is very sensitive to antibiotics. They speculated that the function of the circular molecule may be to code for some component of the photosynthetic process. Roberts and Koths (1976) provided the first direct evidence for the existence of covalently closed circular DNA molecules in *Agmenellum quadruplicatum*. This DNA consists of six or more discrete classes of DNA circles which are physically similar to bacterial plasmids even though they have not yet been shown to replicate autonomously. Their functions remain unknown.

In a more recent study, Van den Hondel *et al.* (1979) have demonstrated the existence of two plasmid species of 5.3 megadaltons and 33 megadaltons in *Anacystis nidulans*. The 5.3 megadalton plasmid contains genes for resistances to streptomycin, ampicillin, and sulphonamide. These workers further found that out of 15 different unicellular blue-greens examined, 14 contained plasmids. These workers are now engaged in making use of these plasmids for genetic engineering and biochemical studies of cyanobacteria.

By now plasmids have been detected in species of *Synechococcus*, *Synechocystis*, *Anacystis*, *Agmenellum*, *Coccochloris*, *Oscillatoria*, *Anabaena*, *Nostoc*, *Calothrix*, and a few other cyanobacteria. These plasmids range in size from 1.9 megadaltons to as large as 75 megadaltons. It has also been found that the base composition of plasmid DNA differs significantly from that of chromosomal DNA (Roberts and Koths, 1976).

Antibiotic resistance genes borne by some transposons or plasmids of *Escherichia coli* have been introduced into a plasmid of *Anacystis nidulans* and hybrid *Anacystis-Escherichia* vectors convenient for gene cloning have been produced (Kuhlemeier *et al.*, 1983; Sherman and Van de Putte, 1982). These vectors are capable of replication in both these organisms. These researches mark a significant milestone in the progress of cyanobacterial molecular biology because these hybrid plasmids act as shuttle cloning vectors. Gendel *et al.* (1983) have produced a shuttle vector of *A. nidulans* and *E. coli* with two antibiotic resistance genes, and a pair of vectors with a single antibiotic resistance gene. These vectors have multiple, unique, closely spaced restriction sites suitable for cloning. It is hoped that the availability of shuttle cloning vectors for use in *E. coli* and cyanobacteria will facilitate the understanding of gene regulation and developmental processes in cyanobacteria. Another type of study likely to be facilitated is that pertaining to problems of chloroplast genes in eukaryotic algae and higher plants.

Mazur *et al.* (1980) have clearly shown that the plasmids of *Anabaena* do not carry any identifiable *nif*-genes.

In some cases, the plasmids of different cyanobacteria show some homology. Such interplasmic homology may even exist among plasmids of different sizes. Since such regions of homology are not confined to single restriction enzyme digest fragments, the regions of homology may correspond to the transposons carried by many bacterial plasmids.

During the last few years, some sequence-specific endonucleases (restriction



enzymes) have also been characterized in *Agmenellum*, *Microcoleus*, *Anabaena*, and *Mastigocladus* (Herdman, 1982; Duyvesteyn *et al.*, 1983).

### THE HETEROCYST

Many filamentous cyanobacteria, when grown aerobically in the absence of fixed nitrogen, form certain morphologically distinct, functionally specialized cells called heterocysts. Heterocysts can be distinguished from vegetative cells by one or two pores. A heterocyst has a thick envelope formed external to the cell wall. A heterocyst is connected to adjacent vegetative cells by one or two pores. It contains vast plugs of homogeneous material at both poles, and it either lacks "nuclear material" or contains much less of it as compared to vegetative cells (Haselkorn, 1978).

The heterocyst envelope consists of three layers (Fig. 2-3), lying external to the outer membrane of the vegetative cell from which the heterocyst differentiated. The outermost layer of the envelope is fibrous, the central layer homogeneous, and the inner laminated. Chemically, the laminated layer is made up entirely of certain glycolipids, the central layer is made of a network of oligosaccharides and polysaccharides with repeating units of glucose-glucose-glucose-mannose. The chemical composition of the outer layer is unknown; it may also contain uncompacted strands of the same polysaccharide as present in the central layer (Wolk, 1982). The glycolipid layer covers the heterocyst protoplast except at the junctions to adjacent vegetative cells. Its function seems to be to reduce the rate of diffusion of oxygen into heterocysts. It acts as a permeability barrier and is involved in the differential gas flow (i.e., the heterocyst is permeable to nitrogen but impermeable to oxygen—see Adams and Carr, 1981). The polysaccharide layer of the envelope possibly protects against degradation of the glycolipid layer and, in addition, hinders the ingress of oxygen into the heterocyst (Wolk, 1982). The glycolipids found in heterocyst envelope are absent in the vegetative cell walls.

The cell membrane of the heterocyst also differs from the plasmalemma of vegetative cells in one important respect: the vegetative cell membrane contains certain 5.5 nm particles which are usually absent or rare in the heterocyst membrane (Giddings and Staehelin, 1978).

Heterocysts contain one or two polar granules made of cyanophycin. They seem to lack DNA and contain much less of phycocyanin than do vegetative cells. They show higher respiratory activity than vegetative cells but have a reduced ability to fix  $\text{CO}_2$ .

The functional significance of heterocysts remained enigmatic for a long time but is now known. They function as anaerobic factories for nitrogen fixation under externally-aerobic conditions. While non-heterocystous species may also fix nitrogen when these organisms grow under near-anaerobic conditions, only the heterocystous forms fix nitrogen when the organism is growing aerobically. Thus, heterocysts are essential for aerobic fixation of nitrogen. In heterocystous filamentous cyanobacteria, the ability to carry out the initial fixation of carbon and nitrogen is segregated into vegetative cells and heterocysts. The vegetative cells contain ribulose biphosphate carboxylase and other enzymes of the Calvin cycle and, hence, fix  $\text{CO}_2$ . Heterocysts lack these enzymes and also lack Photosystem II activity, and hence cannot fix  $\text{CO}_2$ . In aerobic filaments, nitrogenase, which mediates the reduction of dinitrogen to ammonia, occurs mainly in the heterocysts. The heterocysts provide an



anaerobic environment for the protection of nitrogenase which would otherwise be inactivated in the presence of oxygen. They do this by means of their three-layered envelope which blocks or reduces the entry of gases into the heterocysts. Since they lack PS-II, no oxygen is evolved within the heterocyst. Some transport of reduced sulphur compounds, e.g., sulphide, occurs from vegetative cells into heterocysts (Giddings *et al.*, 1981) and this helps maintain anaerobiosis within the heterocysts. At the same time, heterocysts do have PS-I which generates the ATP needed to fix nitrogen. The nitrogen fixed in the heterocysts, probably in the form of glutamine, is transported to vegetative cells (Thomas *et al.*, 1977). In the opposite direction, the flow of carbon compounds from vegetative cells to heterocysts is ensured by the establishment of pumping devices at the junctures of the heterocysts with the neighbouring vegetative cells; these carbon compounds serve as reductants for the nitrogenase. The biochemical bases underlying the pumps are at present unknown (Haselkorn, 1978).

Gupta and Carr (1981) have reported that enzymes catalyzing the synthesis and breakdown of cyanophycin occur plentifully within heterocysts. In fact, cyanophycinase and nitrogenase are the two most abundant enzymes in heterocysts. Adams and Carr (1981) have concluded that in heterocysts arginine is actively synthesized and that cyanophycin has a rapid turnover (being synthesized and broken down).

As compared to vegetative cells, the distribution of DNA is more diffuse in heterocysts. Simon (1980) has shown that a heterocyst of *Anabaena variabilis* contains more RNA and nearly as much DNA as a vegetative cell.

Many workers have examined the various stages in the development of a vegetative cell into heterocyst (Wildon and Mercer, 1963; Lang, 1965; Lang and Fay, 1971; Stewart, 1972). These involve the deposition of the additional wall layers in the sequence of fibrous, homogeneous, and inner laminated layer, development of pores at one or both poles, formation of plasmodesmata which connect the plasmalemmas of the heterocyst and the adjacent vegetative cells (Wildon and Mercer, 1963; Lang and Fay, 1971), the secretion of polar granules, gradual disappearance of cytoplasmic granules, and reorientation of the photosynthetic thylakoids into a honeycomb-like pattern at the polar regions (Lang, 1965). The heterocysts also lack gas vacuoles. In some species, the above developmental stages may be telescoped or arrested at some stage, leading to incomplete differentiation of heterocysts (Fay *et al.*, 1964; Whitton and Peat, 1967). In some species, an intermediate stage, called proheterocyst, between vegetative cell and mature heterocyst, may be distinguishable. The proheterocyst lacks the thickened cell wall and refractile polar granules characteristic of the mature heterocyst, and it can be distinguished from the vegetative cell by its more regular shape and less granular contents. Normally, proheterocysts mature into heterocysts but under certain abnormal conditions they may revert back to vegetative cells. In *Anabaena catenula*, *A. cylindrica*, and *A. azollae*, one can observe seven stages between the proheterocyst and the mature heterocyst (Wolk, 1982). These are: (1) development of a thin fibrous envelope layer around the cell, (2) completion of the fibrous envelope, and decrease in the diameter of the junctions with adjoining cells, (3) some elongation of the cell, accompanied by development of the homogeneous envelope layer, (4) further deposition of the homogeneous layer, and assumption of the final shape of the protoplast in the region of the pore channel,



(5) deposition of the laminated layer of the envelope near the poles of the cell, (6) formation of pore-plugs, and (7) extensive proliferation of photosynthetic thylakoids, resulting in mature heterocyst (Wolk, 1982). During this series of changes, glycogen granules and polyphosphate granules decrease in number, and carboxysomes are lost, but the original cell wall of the mother cell is retained.

Adams and Carr (1981) have reported that the thylakoid arrangement of heterocysts differs from that of vegetative cells. In the centre of the heterocyst, thylakoids are more openly coiled and are not pressed adjacent to each other, as in vegetative cells. But in the polar regions, the thylakoids are tightly coiled.

Table II shows an enzymological comparison between heterocysts and vegetative cells.

**Table II** Enzymological comparison between heterocysts and vegetative cells of *Anabaena cylindrica* (grown aerobically) (after Wolk, 1982)

Activity mainly or entirely in heterocysts	Activity mainly or entirely in vegetative cells
ENZYMES	ENZYMES
Nitrogenase	Glutamate synthase
Cyanophycinase	Ribulose biphosphate carboxylase
Glucose-6-phosphate dehydrogenase	oxygenase
6-Phosphogluconate dehydrogenase	Ribulose-5-phosphate kinase
'Uptake' hydrogenase	Phosphoglycollate phosphatase
Isocitrate dehydrogenase	Fructose-1,6-diphosphate aldolase
Arginyl polyaspartate synthetase	Glycollate dehydrogenase
	3-Phosphoglycerate kinase
	Phosphofructokinase
	Pyruvate kinase
MAIN METABOLIC PATHWAYS	MAIN METABOLIC PATHWAYS
Nitrogen fixation	Reductive pentose phosphate cycle
Oxidative pentose phosphate cycle	Photosystem II

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# 3 Cell Structure—Eukaryotic

## INTRODUCTION

Unlike Prokaryota, the cells of eukaryotic algae (Figs. 3-1, 3-2) typically have membrane-bound discrete nuclei, chromatophores, mitochondria, and Golgi bodies. Their cell walls do not contain mucopeptides. These algae have their cells dividing by mitosis, and most species undergo true sexuality comprised of alternating karyogamy (syngamy) and meiosis. They have typical chromosomes in which histones are associated with nucleic acids.

All algae (except the blue-greens and Prochlorophyceae) are eukaryotic. The systematics and classification of the eukaryotic algae have presented many problems particularly in the era of electron microscopy when several hitherto unknown aspects of their structure and organization have become known. No single acceptable system of classification has emerged. The presently available systems keep on changing as more and more new information or knowledge is generated. The number of classes tends to increase with the passage of time.

In this chapter, we give brief descriptions of the general cell structure and cytology of several classes whose number, sequence, and nomenclature are arbitrary and do not necessarily imply any phylogenetic relations. Table III lists the main photosynthetic pigments recorded in different classes (as far as are known).

## CHLOROPHYCEAE

The Chlorophyceae *sensu* Fritsch has been split up into a number of classes by various algologists during the last two decades, largely as a consequence of new discoveries about their fine structure. Round (1971) split up the phylum Chlorophyta into four classes termed the Chlorophyceae, Zygnemaphyceae, Oedogoniophyceae, and Bryopsidophyceae. Pickett-Heaps (1975) and Stewart and Mattox (1975) divided the green algae into the Charophyceae and Chlorophyceae mainly on the basis of cytological features. Their class Charophyceae characteristically has a persistent interzonal spindle at telophase, has motile cells in which the flagella are associated with a single band of microtubules, and their cells have the enzyme glycollate oxidase. The motile cells, when present, are covered with minute, diamond-shaped organic scales. Typical examples of this class are *Coleochaete*, *Chara*, *Klebsormidium*, and *Spirogyra*. The second class, Chlorophyceae, in contrast, has a non-persistent (collapsing) interzonal spindle at telophase, has motile cells in which the flagellar apparatus has four or more narrow microtubular roots, and the cells lack glycollate oxidase. In the Charophyceae, cytokinesis is effected by a phragmoplast which arises from the persistent spindle. In the Chlorophyceae, a transverse system of microtubules, called phycoplast, develops between the nuclei and seems to facilitate the development of the new septum which may either develop as a furrow (*Tetradron*) or as a cell plate (e.g., *Oedogonium*).

Melkonian (1980, 1981) classifies the green algae into three classes, relying mainly on ultrastructural characteristics of motile cells, mitosis, and cytokinesis. The three classes are the Charophyceae, Chlorophyceae, and Ulvophyceae (some



workers spell it as Ulvophyceae). The Ulvaphycean motile cells also have small organic scales but these are lacking in the Chlorophyceae. In both the



Fig. 3-1 Electron micrograph of longitudinal section through the dinoflagellate *Woloszynskia tylosa*, showing a typical eukaryotic structure. The nucleus, chromophores, thecal cell covering, girdle, and other organelles can be seen. (x5600.) Courtesy J.D. Dodge.

Chlorophyceae and Ulvaphyceae, the motile cells have flagella that are apically attached and associated with a cruciate (x-2-x-2) flagellar root system, but the detailed structure of the flagellar apparatus differs in the two classes.

Recently, Ettl (1981) has created yet another class, called Chlamydomonadeophyceae, whose motile cells are covered by a cell wall made of crystalline glycoprotein. However, this proposal does not seem to be justified in view of the fact that even



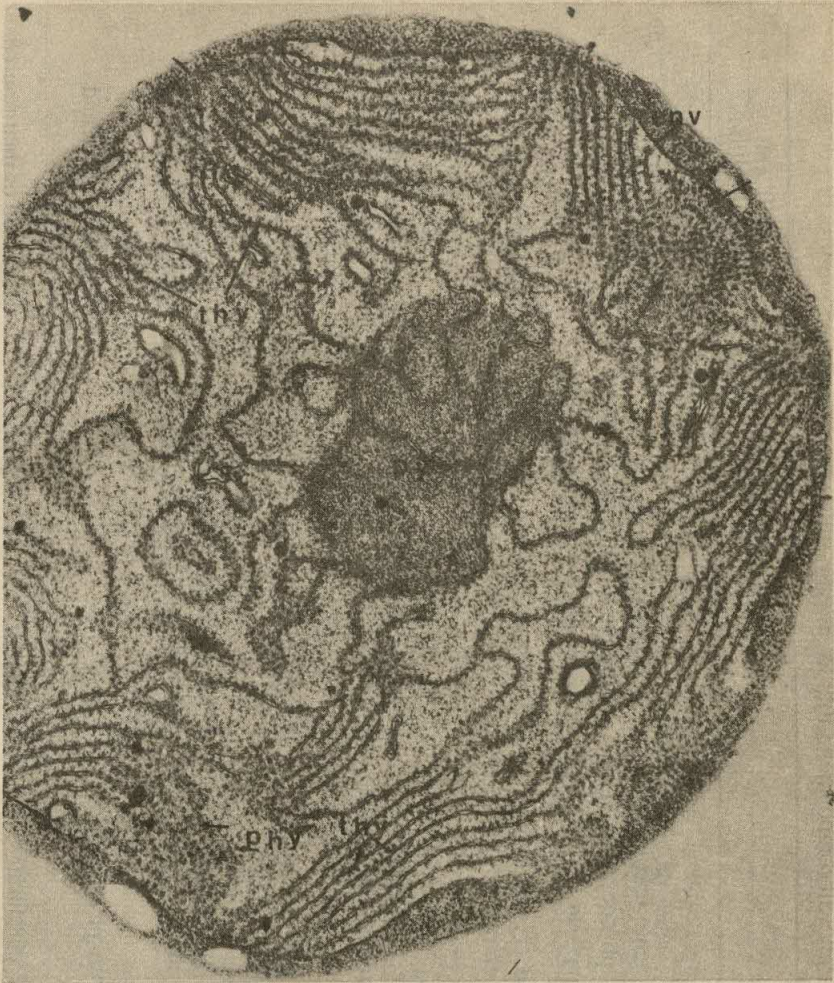


Fig. 3-2 Electron micrograph of section through the red alga *Porphyridium cruentum*. The cell volume is seen to be mostly occupied by the chromatophore having a double-membraned envelope, a central pyrenoid, and a granular matrix of double discs covered with phycobilisomes. env, envelope; th, thylakoids discoidal; phy, phycobilisomes; py, pyrenoid. (x27,100.) Courtesy M. Lefort-Tran.

some members of the Chlorophyceae can produce naked motile cells that are virtually indistinguishable from those of the Chlamydomyceae.

Although the green algae may be split up into 2, 3, 4, or more groups cytologically, their physiological and biochemical characteristics are fairly identical. They have chlorophylls a and b, beta-carotene (with lesser amounts of alpha- and gamma-carotene), and several xanthophylls, e.g., lutein, zeaxanthin, astaxanthin, siphonein, siphonaxanthin, and violaxanthin. Starch is their main food reserve. Their cell walls predominantly contain cellulose. Their cells have one or more nuclei, chloroplasts, pyrenoids, mitochondria, Golgi bodies, endoplasmic



**Table III** Algal pigments, food reserves, and cell wall composition (after Rosowski and Parker, 1982)

Class	Pigments*	Food reserves	Cell wall composition
<b>PROKARYOTIC</b>			
Cyanophyceae	Myxoxanthophyll, $\beta$ -carotene, zeaxanthin, echinone, oscillaxanthin, phycocyanin, allophycocyanin, phycoerythrin	Polyglucans (glycogen), cyanophycin, polyphosphate, occasionally polyhydroxybutyrate	Muramic acid
Prochlorophyceae	Chlorophyll b, $\beta$ -carotene, cryptoxanthin (biliproteins are absent)	Glycogen, amylose	Muramic acid
<b>EUKARYOTIC</b>			
Chlorophyceae	Chlorophyll b, $\beta$ -carotene, zeaxanthin, lutein, violaxanthin, neoxanthin, sometime siphonoaxanthin	Amylose plus amylopectin (starch)	Cellulose; some protein
Euglenophyceae	Chlorophyll b, and other pigments are as in Chlorophyceae	Paramylum	
Rhodophyceae**	$\beta$ -Carotene, zeaxanthin, phycoerythrin, phycocyanin, allophycocyanin	Floridean starch, floridoside, trehalose, isofloridoside, maltose, sucrose	Cellulose, xylans, galactans, $\text{CaCO}_3$
Cryptophyceae	Chlorophyll c, phycocyanin, phycoerythrin (allophycocyanin absent)	Starch	Fibrous periplast and some protein
Dinophyceae	Chlorophyll c, $\beta$ -carotene, peridinin, dinoxanthin	Oil, starch	Armoured or unarmoured, theca or amphiesma of various compositions



**Table III** Algal pigments, food reserves, and cell wall composition (after Rosowski and Parker, 1982) (cont.)

Class	Pigments*	Food reserves	Cell wall composition
Chrysophyceae	Chlorophyll c, $\beta$ -carotene, several xanthophylls, mainly fucoxanthin	Chrysolaminarin, oil	Cellulose, chitin, siliceous scales
Bacillariophyceae	Chlorophyll c, $\beta$ -carotene, fucoxanthin, diadinoxanthin, diatoxanthin, neofucoxanthin	Chrysolaminarin, lipids, some glucan	Silica
Prymnesiophyceae	Chlorophyll c, $\beta$ -carotene, fucoxanthin		
Chloromonadophyceae	Chlorophyll c, $\beta$ -carotene, antheraxanthin, lutein	Oil	Coccoliths, scales Cell wall absent
Tribophyceae	Chlorophyll c***, $\beta$ -carotene, diatoxanthin, heteroxanthin, vaucheriaxanthin ester	Chrysolaminarin, oils, fats, mannitol, glucans, xylans	Cellulosic, of two overlapping halves
Eustigmatophyceae	$\beta$ -Carotene, violaxanthin, vaucheriaxanthin ester	Some refractile carbohydrate	Cellulose ?
Phacophyceae	Chlorophyll c, $\beta$ -carotene, fucoxanthin	Laminarin, mannitol	Cellulose, alginate, fucoidin
Prasinophyceae	Chlorophyll b, and other pigments are as in Chlorophyceae; some have siphonein and siphonaxanthin instead of lutein	Starch, mannitol	Pectic material (galactose, galacturonic acid, arabinose), organic scales

\*All classes have chlorophyll a.

\*\*Chlorophyll d was once reported in some red algae but has not been confirmed.

\*\*\*Discovered recently in *Vaucheria* and some other taxa; earlier it was believed to be chlorophyll e.



reticulum, microbodies, and motile cells also have eyespots. Detailed descriptions of these and other organelles (e.g., flagella) are given in subsequent chapters.

The belief of many early botanists (see Bower, 1908) that higher green plants (e.g., the bryophytes) have evolved from ancestral green algae has received strong support from biochemical and ultrastructural studies on diverse members of the class Charophyceae, particularly *Coleochaete* spp. (Graham, 1984). *Coleochaete* has a phragmoplast, peroxisomes of the land-plant type (Marchant and Pickett-Heaps, 1974), and glycollate oxidase. Its zoospores have a multilayered structure resembling a similar structure present in the spermatozooids of land plants. *Coleochaete* antheridia resemble early developmental stages of the sunken antheridia characteristic of the Anthocerotales and *Equisetum*. *Coleochaete* is unique among green algae and similar to embryophytes in that its zygote is retained on the parental thallus and undergoes further cell divisions there. Indeed, the zygotes of prostrate species (e.g., *C. scutata*) are firmly embedded in the mother plant. All these attributes have provided strong support for Bower's (1908) views on the origin of land plants.

The Chlorophyceae is a very wide class, ranging from unicellular to elaborately differentiated, heterotrichous filamentous algae. Colonial, filamentous, palmeloid, dendroid, coenocytic, and siphonous forms as also parenchymatous and pseudoparenchymatous forms occur in this group.

### XANTHOPHYCEAE (TRIBOPHYCEAE)

As compared to the isokont Chlorophyceae, the Xanthophyceae were formerly known as Heterokontae because of their unequal flagella; their modern name is Tribophyceae (Hibberd, 1981). They show much parallelism in the range of thallus structure with the Chlorophyceae. Thus, the order Volvocales of the Chlorophyceae *sensu* Fritsch (Fritsch, 1935) corresponds to the Heterochloridales of the Xanthophyceae, Chlorococcales corresponds to Heterococcales, Ulotrichales to Heterotrichales, and Siphonales to Heterosiphonales. Pigmentation is broadly similar to that of the Chlorophyceae but chlorophyll b is lacking, and some members may contain what is known as chlorophyll e (though this remains unconfirmed). Chlorophyll c is known to occur in many but not all members. Apart from beta-carotene, the other pigments are diatoxanthin, diadinoxanthin, and heteroxanthin. The food reserves are chrysolaminarin, lipids, mannitol, 1,6-linked glucan, and 1,4- and 1,3-linked xylans.

Cell structure is fairly simple with one or more nuclei, chloroplasts, Golgi bodies, mitochondria, ribosomes, and in motile cells, eyespots. The cell walls of many genera occur as H-shaped (2-part, overlapping) pieces. Pyrenoids are rare but, when present, occur in vegetative cells as well as motile stages. Asexual reproduction commonly involves autospore formation or occurs by means of heterokont biflagellate zoospores. Sexual reproduction is rather rare but advanced oogamy is prominent in *Vaucheria*.

*Botrydium*, *Tribonema*, and *Vaucheria* are three representative genera.

### EUSTIGMATOPHYCEAE

On the basis of electron microscopic studies, Hibberd and Leedale (1970) proposed that some algae until then included in the Xanthophyceae should be transferred to a new class called the Eustigmatophyceae, because of their unusual and unique organization of motile cells (Fig. 3-3). This new class, created almost entirely on the basis of ultrastructural aspects, is characterized collectively by the following features:



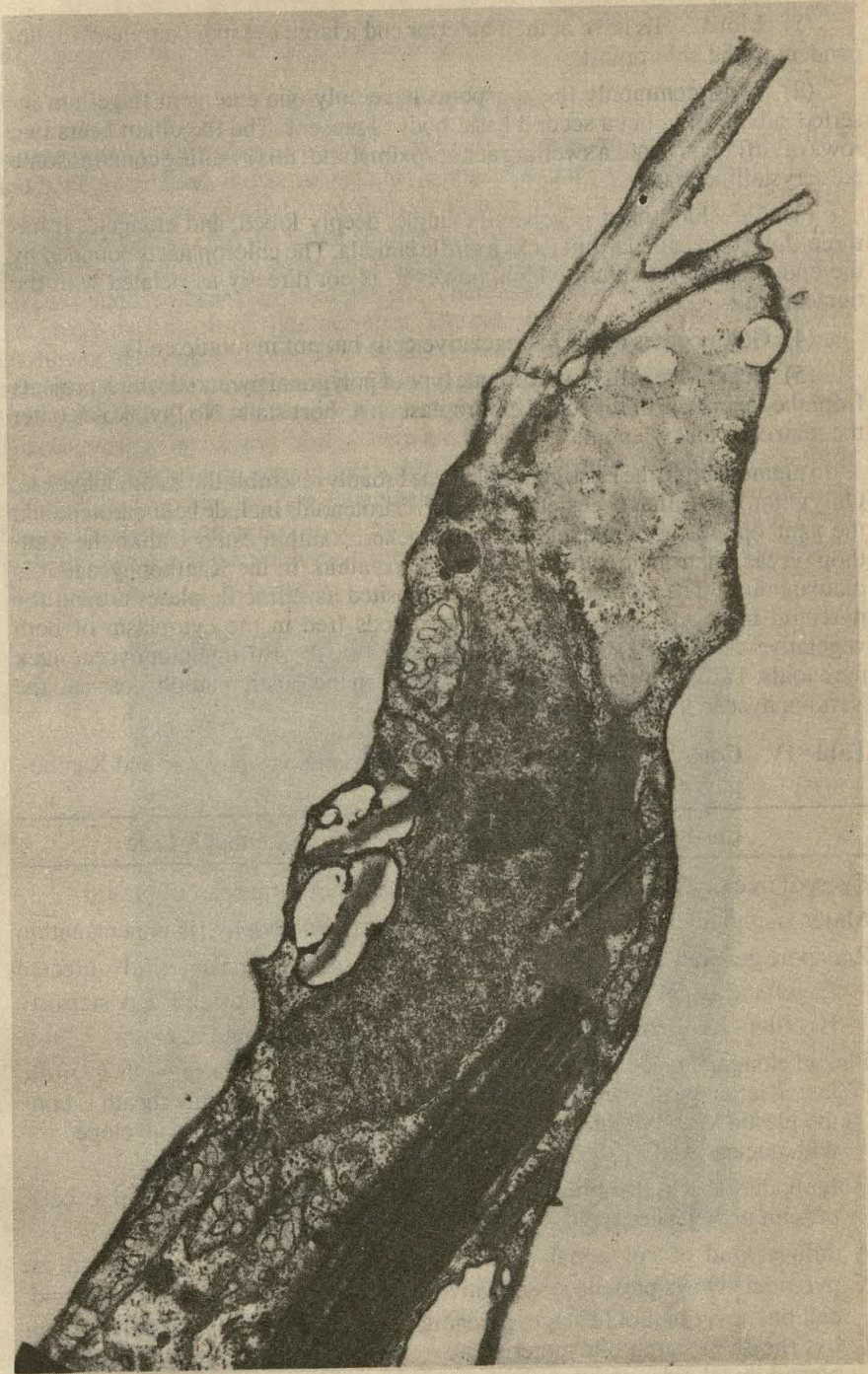


Fig. 3-3 Electron micrograph of longitudinal section through *Eustigmatos*, illustrating the typical Eustigmatophycean cell structure. (x20,000.)  
Courtesy D.J. Hibberd.



(1) Motile cells have at their anterior end a large eyespot completely independent of the chloroplast.

(2) Most commonly the zoospores have only one emergent flagellum inserted subapically, but a second basal body is present. The flagellum bears two rows of stiff hairs. It has a swelling at its proximal end; this swelling contains some paracrystalline material.

(3) The chloroplast is generally single, deeply lobed, and elongate. It has three-thylakoid lamellae but lacks a girdle lamella. The chloroplast is bounded by the endoplasmic reticulum which, however, is not directly associated with the nuclear wall.

(4) Golgi bodies occur in vegetative cells but not in motile cells.

(5) Vegetative cells have a unique type of polygonal pyrenoid which projects from the inner face of the lobed chloroplast on a short stalk. No thylakoids enter the matrix of the pyrenoid.

In pigmentation, the Eustigmatophyceae broadly resemble the Xanthophyceae. Only chlorophyll a has been detected so far. Carotenoids include beta-carotene and the xanthophylls are violaxanthin and vaucherixanthin ester. Unlike the Xanthophyceae, their main xanthophyll is violaxanthin (in the Xanthophyceae it is diatoxanthin). The storage material is deposited as refractile plates around the pyrenoid in vegetative cells and as short rods free in the cytoplasm of both vegetative cells and zoospores. Motile stages of the Eustigmatophyceae lack pyrenoids. Table IV lists the differences between the Eustigmatophyceae and the Xanthophyceae.

**Table IV** Comparison of main characters of Eustigmatophyceae and Xanthophyceae

Eustigmatophyceae	Xanthophyceae
Eyespot free of plastid	Eyespot forms part of plastid
Major xanthophyll is violaxanthin	Major xanthophyll is antheraxanthin
Zoospore generally has only 1 emergent flagellum; flagellar insertion subapical; flagellum has 2 rows of stiff hairs	Zoospore has an anteriorly directed hairy flagellum and a posteriorly directed smooth flagellum
Plastid elongate, made of three-thylakoid lamellae; no girdle lamella; ER bounds the plastid but is not directly connected with nuclear wall	Plastids 2 or more with a girdle lamella; plastid ER sheath is continuous with nuclear envelope
Golgi bodies absent in motile stages but present in vegetative cell	Both motile and vegetative cells have Golgi bodies
A unique kind of polygonal projecting pyrenoid always present in vegetative cell but never in motile stage; pyrenoid has sheath of starch-like material; no plastid thylakoids enter pyrenoid	Pyrenoids rare but, when found, are present in both vegetative cell and zoospore; no stalk, no polysaccharide sheath; plastid lamellae enter pyrenoid
Examples: <i>Pleurochloris</i> , <i>Polyedriella</i> , <i>Vischeria</i>	Examples: <i>Botrydium</i> , <i>Tribonema</i> , <i>Mischococcus</i>



Some representative genera belonging to the Eustigmatophyceae are *Eustigmatos*, *Chlorobotrys*, *Vischeria*, and *Monodopsis*.

### EUGLENOPHYCEAE

Most euglenoids are photosynthetic unicellular algae having both chlorophylls a and b, beta-carotene, lutein, neoxanthin, antheraxanthin, and astaxanthin. These pigments occur in discoid, rod-shaped, ribbon-like, or stellate chloroplasts. Paramylum and oil are the usual storage reserves, with paramylum (a beta-1,3-linked glucan) occurring as several discrete granules in the cells. Phospholipid vesicles and lysosomes are also present. There is no cellulosic cell wall and the cells have only a soft or rigid periplast. The cell surface is often helical and has knobby or warty projections. The organisms having a soft pellicle, e.g., *Euglena*, exhibit a characteristic contractile (flowing) type of motility, whereas those having firm pellicle (e.g., *Phacus*) have a fixed shape and lack flexibility. Cells of *Trachelomonas* and a few other genera are loricate.

Cells of euglenoids are anteriorly biflagellate but only the longer flagellum emerges out from the anterior invagination (Fig. 3-4), whereas the other, shorter flagellum stops short or is non-emergent. The shorter flagellum is generally fused with the basal part of the long flagellum, where a knot-like structure, named flagellar swelling, can be seen.



Fig. 3-4 Scanning electron micrograph of *Euglena gracilis*, showing the emergent flagellum. (x2300.)

Courtesy M. Lefort-Tran.

The nuclear characteristics, mitosis, and eyespots of euglenoids are quite interesting (see Osafune and Schiff, 1980) and are described in subsequent chapters.



## CHRYSTOPHYCEAE

These have chlorophylls a and c, beta-carotene, fucoxanthin, cryptoxanthin, violaxanthin, and zeaxanthin. They commonly appear golden or golden brown in colour. Their food reserves are lipid droplets, leucosin, and certain polyglucans with beta-1-3 and beta-1-6 linkages. Photosynthetic cells possess 1 or 2 golden chromatophores surrounded by chloroplast ER; a periplastidal reticulum is present between the latter and the plastid envelope. Plastids have 3-thylakoid lamellae and usually a girdle lamella. Non-photosynthetic members have a leucoplast likewise surrounded by ER cisternae. Some chrysophytes, e.g., *Chrysosphaerella brevispina* and *Spiniferomonas cornuta*, possess eyespots.

Members are unicellular or colonial flagellates. Cells are anteriorly biflagellate, centrally uninucleate, with two lateral chloroplasts. Cells have two unequal, heterodynamic flagella one of which typically possesses two rows of tubular hairs. A single nucleus lies posterior to the flagellar base. A single Golgi body is present near the flagellar base. Mitochondria contain tubular cristae. Cell membrane is generally covered with silica scales or may be loricate (Glider and Rosowski, 1976).

One very characteristic feature of the class is the ability to form endogenous silicified resting stages called stomatocysts.

Chrysophytes mainly reproduce asexually, but sexual reproduction is known for several species now. Some representative genera of this class are *Mallomonas*, *Ochromonas*, and *Dinobryon*.

## PRYMNESIOPHYCEAE (HAPTOPHYCEAE)

This class has been carved out from the Chrysophyceae with which it resembles in pigmentation and food reserves. It differs from the Chrysophyceae in respect of flagellation, chloroplast and eyespot characters, and some aspects of the cell wall. The Prymnesiophyceae commonly have two anterior, equal and smooth flagella plus a characteristic flagellum-like appendage, the haptonema, also inserted anteriorly. The haptonema has a sticky tip by means of which the alga attaches itself to the substratum.

The Prymnesiophyceae include some algae which bear organic scales, and also the coccolithophorids which form calcite scales or coccoliths. Basically, a Haptophycean cell contains two chloroplasts, one nucleus, one Golgi body, and a few mitochondria (Fig. 3-5). Several types of pyrenoid, e.g., immersed, bulging, or projecting, occur in different (sometime the same) species. Many genera show phagotrophy (i.e., ingest particulate organic matter).

The order Pavloales has been extensively studied in recent years. These members have distinctive features not found in other members of the Prymnesiophyceae. These features of the Pavloales are heterokont flagella, presence of knob-scales on the longer flagellum and occasionally also on the haptonema and the cell itself, a canal entering the cell close to the flagellar insertion and penetrating deeply (Green, 1980).

Examples of the Prymnesiophyceae are *Chrysochromulina*, *Hymenomonas*, *Prymnesium*, and *Pavlova*.

## BACILLARIOPHYCEAE

Popularly called diatoms, the most characteristic feature of these algae is the formation of a silica wall or frustule. Diatoms are predominantly unicellular but some are colonial or filamentous. They possess chlorophylls a and c, beta-





Fig. 3-5 Electron micrograph of section through cell of *Cricosphaera roscoffensis*. ( $\times 13,000$ .) Courtesy P. Gayral.

carotene, fucoxanthin, diatoxanthin, and diadinoxanthin. Leucosin and oil are their main storage reserves. Motile stages have a single flagellum with stiff hairs.

Cells are uninucleate, with two or more chloroplasts, several Golgi bodies, and mitochondria. In most diatoms, the dictyosomes are localized exclusively in the plasma packet containing the nucleus, but in *Synedra* they lie in a plasmatic ribbon-like structure extending over the whole length of the filament. The nuclear division of a diatom was well investigated in the nineteenth century by Lauterborn.



Characteristic spindles, called central spindles, appear in mitosis. Recently, the central spindles have been investigated with an electron microscope.

Cells of *Eunotia* and certain other diatoms have conspicuous chromocentres, and the extent of development of constitutive heterochromatin is exceptionally high in *Navicula radiosa* and *N. gracilis* (see Geitler, 1979). In these species, the heterochromatin forms a giant, central, compound chromocentre. However, this extensive chromocentre becomes dispersed and loose in the auxospores.

Examples of diatoms are *Pinnularia*, *Cocconeis*, *Asterionella*, and *Navicula*.

### PRASINOPHYCEAE

The flagellates included in this class previously used to be placed in the order Volvocales *sensu* Fritsch. Ultrastructural studies revealed the existence of some unusual and novel features which led to the removal of the prasinophytes from the Chlorophyceae and to the creation of the class Prasinophyceae (Christensen, 1966). Prasinophytes may be motile, coccoid, or palmelloid, having pigments similar to those of the Chlorophyceae. They share the following characters:

(1) Four or less flagella arise from an apical pit. Flagella have a coating of scales and hairs. They are thicker than those of the Chlorophyceae. The flagellar scales are arrayed in two layers in some species, e.g., *Prasinocladus marinus*. Flagella are equal in length and anteriorly inserted.

(2) The motile cell is covered with one to several characteristic layers of organic scales. Some species have a flexible, non-cellulosic theca in place of the scales. *Pyramimonas tetrarhynchus* has six different types of scale and one cell has 370,000 scales (Moestrup and Walne, 1979). Scales may sometime be siliceous rather than organic.

(3) A special kind of fibrous band connects the flagellar bases to the chloroplast and nuclear envelope.

(4) The pyrenoid is penetrated by extensions from the cytoplasm or even the nuclear envelope.

(5) The contractile vacuoles situated at the anterior end empty into the apical depression.

(6) A pair of prominent dictyosomes is found above the nucleus on either side of the flagellar bases.

(7) Flagellar scales and hairs are produced in a scale reservoir, whereas the body scales originate in vesicles of the Golgi apparatus.

(8) The eyespot forms part of the chloroplast.

(9) Zoospores tend to be formed in pairs.

(10) Cell proliferation occurs by longitudinal division and sexual reproduction is mostly unknown.

(11) Mannitol is an important food reserve besides starch and lipid droplets. However, unlike the Chrysophyceae, sucrose is absent (Ragan and Chapman, 1978).

This class comprises a heterogeneous assemblage of algae, especially in so far as the types of mitosis and cytokinesis are concerned. Thus, *Pedinomonas*, *Pyramimonas*, and *Nephroselmis* show a persistent spindle similar to that found in the Charophyceae, whereas a collapsing mitotic apparatus and phycoplast resem-



bling those of the Chlorophyceae are encountered in *Platymonas* (Mattox and Stewart, 1977). This kind of cytological heterogeneity does not warrant a distinct class status for the prasinophytes, and future work may lead to dismemberment of the Prasinophyceae and redistribution of its genera to different classes. Some recent work of Norris (1980), however, seems to strengthen the integrity of the class in view of the presence of an evolutionary series among these flagellates.

The representative genera of this class have been mentioned above.

### CRYPTOPHYCEAE

Popularly called cryptomonads or cryptophytes, these unicellular flagellates are quite frequently the most abundant phytoflagellates in freshwater and marine habitats. They are mostly isokont (sometime slightly heterokont) and the two flagella arise from an anterior or lateral depression in the cell. The cells are generally ovoid and dorsiventrally flattened. They appear rather simple in structure under a light microscope but, at the fine structural level, are quite complex. Asexual reproduction occurs by cell divisions, and sexual reproduction is not known so far.

Their pigments include chlorophylls a and c, phycobilins (phycocyanin and phycoerythrin), alpha- and epsilon-carotene, cryptoxanthin, and zeaxanthin. Unlike other groups of algae having chlorophyll c, in cryptomonads the periplastidal compartment (i.e., the space between the plastid envelope and plastid ER) is much more extensive and contains starch grains, ribosomes, and a nucleomorph in addition to the vesicles and tubules observed in other algae containing plastid ER (Gillott and Gibbs, 1980). Most species of the Cryptophyceae examined seem to contain one nucleomorph per cell but this number becomes two during cell division. The fact that one nucleomorph is present in each periplastidal compartment (Gillott and Gibbs, 1980) suggests its possible role as a subsidiary genome. This view is strengthened by the observation of division stages (Hibberd, 1977) and the presence of DNA in the nucleomorph (Gillott and Gibbs, 1980). The nucleomorph in interphase cells may be embedded in an invagination of the pyrenoid, or it may lie along the inner surface of the chloroplast. The nucleomorph was first discovered by Greenwood *et al.* (1977) and was thought to represent the rudimentary nucleus of a eukaryotic endosymbiont, probably an ancient red alga that in turn had acquired its plastids through endosymbiotic cyanobacteria.

Cryptomonads are enclosed by a distinctive periplast made up of proteinaceous plates lying just beneath the plasmalemma (Gantt, 1980).

Several genera are animal-like in their morphology and nutrition with the cell surface near the flagellar base being invaginated to form a large gullet which, especially in colourless and saprophytic forms, functions as a means of ingestion of food particles. The apical or subapical gullet has a tubular invagination and a narrow aperture (Santore, 1982). It may be divided into a vestibular region (where the flagella are inserted) and a trichocyst-lined channel extending posteriorly into the cell. The contractile vacuoles discharge into the vestibular region. In *Chroomonas mesostigmatica*, however, Santore has shown that the gullet branches below the vestibule form two channels: (1) one lined with trichocysts and (2) the other going toward the eyespot (Santore, 1982). Cryptomonads have a characteristic flagellar rootlet system which is different from that found in any other group of algae. Like the heterokont algae, however, they do have mastigonemes, but these mastigonemes are present on both the flagella (Ludwig and Gibbs, 1985).



The reserve photosynthate is starch.

Cells typically have two large parietal chloroplasts but some species have several discoid chloroplasts. Several pyrenoid-like structures are present within the cell. Cells also have Golgi bodies, mitochondria, and usually a single contractile vacuole at the anterior end.

Cryptomonads also produce a unique kind of ejectile organelle called trichocyst. In holozoic cryptomonads, trichocysts line the gullet; in other species, they may line the gullet region as well as the cell periphery. Cryptophycean trichocysts act as a means of escape and also as buffers against adverse conditions. Before ejection, the long and short tubes of a trichocyst are coiled into two connected cylindrical structures. In ejected trichocyst, the longer tube is seen joined at an angle with the shorter tube. Both the tubes are tapered. Trichocysts generally form in the Golgi body but in some cases may be observed in apposition to the endoplasmic reticulum.

Some cryptomonads possess roughly spherical structures, usually two per cell, between the Golgi apparatus and the chloroplast. These structures are called *Corps de Maupas* and are sometime visible even under the light microscope. Functionally, they may represent a food storage body or may be lysosomal (Lucas, 1970).

The mitotic process seems to be unique and is described later. Representative genera besides *Cryptomonas* are also listed later.

### DINOPHYCEAE (PYRRHOPHYTA, DINOPHYTA)

Popularly called dinoflagellates, these predominantly unicellular flagellates are unique in several respects, especially nuclear and chromosomal characteristics.

Their pigments include chlorophylls a and c, beta-carotene, peridinin, dinoxanthin, and diadinoxanthin. Reserve products are stored mainly as starch and oil. A unique feature is the presence of dinosterol (Sigee, 1986). A cellulose cell wall may or may not be present. Motile members have two flagella oriented in two different planes and lying in grooves. One of these is naked and the other bears a single row of long, fine hairs. Cells have eyespots, trichocysts, contractile vacuoles, discoid or band-shaped chloroplasts, a large central vacuole, and a large prominent nucleus (Fig. 3-6). The nucleus contains unusually high DNA content as compared to other eukaryotes. Another characteristic feature of most dinoflagellates is the presence of conspicuous membrane-bound vacuoles, called pusules, which lack contractile activity. Nutritional patterns range from free-living holophytic to holozoic, saprophytic, parasitic, or symbiotic, with most species being photoautotrophic. The dinoflagellates may be armoured (thecate, i.e., having rather thick wall) or unarmoured (non-thecate). The armoured Dinophyceae have their thecae composed of numerous articulated and variously sculptured plates. A few species such as *Oxyrrhis marina*, *Heterocapsa triquetra*, and *Glenodinium* sp. also possess body scales. These generally occur external to the thecal plates. *O. marina* also has flagellar scales (Pennick and Clarke, 1977).

Binary fission is the chief mode of reproduction but sexual reproduction also occurs in many species. The sexual cycle usually has dimorphic stages. Except in *Noctiluca*, the sexual life histories of other dinoflagellates are all haplontic. Some dinoflagellates cause red tides that are toxic to humans and marine animals. Some species are bioluminescent.

Dinoflagellate chromosomes lack histones and can be seen at all stages of the nuclear cycle. When examined with an electron microscope, the chromosomes show a very distinctive and characteristic structure which is not seen in any other



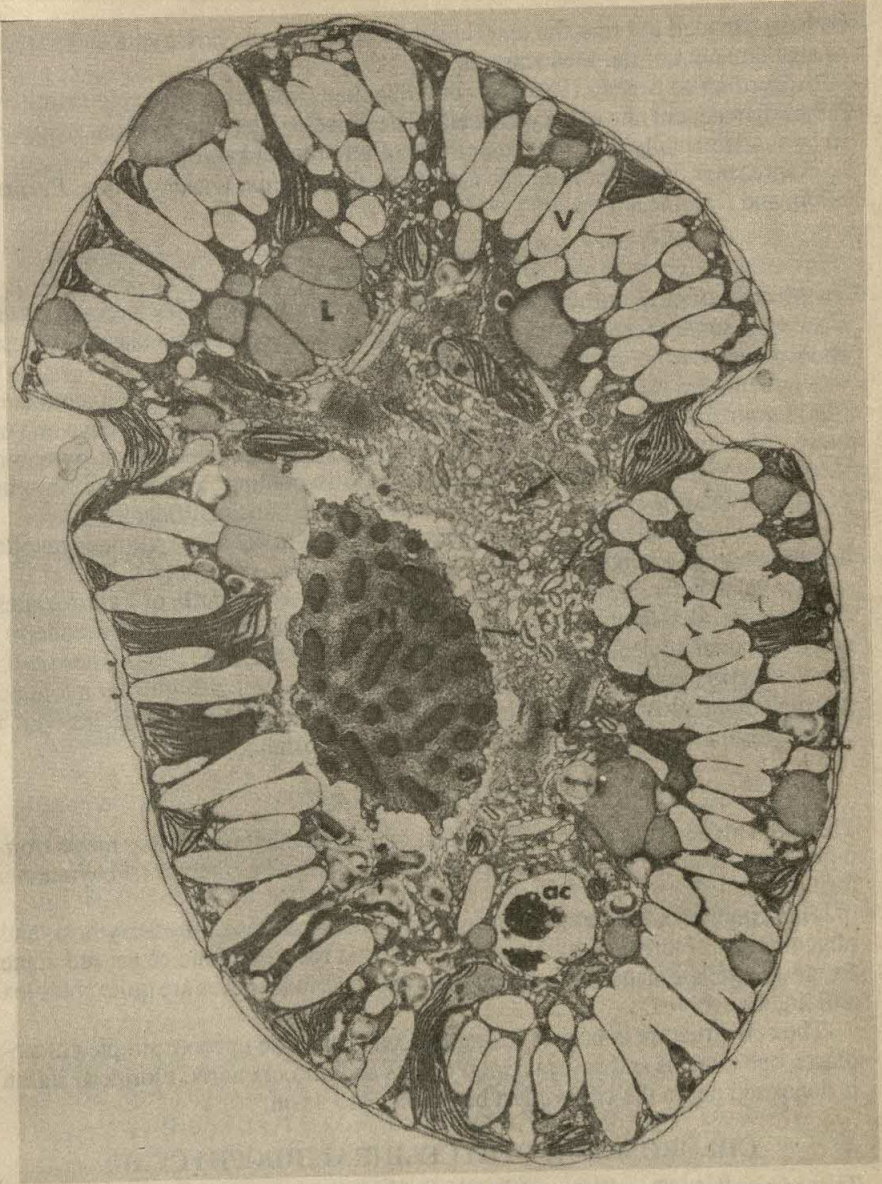


Fig. 3-6 Photomicrograph of section of whole cell of *Amphidinium cryophilum*, showing peripheral arrangement of large vacuoles and chloroplasts. A conspicuous nucleus can be seen. Branched (thick arrows) and unbranched (thin arrows) portions of the pusular tubules are evident. ac, accumulation body; C, chloroplast; d, dictyosome; L, lipid droplet; N, nucleus; s, starch grain; V, vacuole. (x1300.) Courtesy L.W. Wilcox.

algal chromosomes.

Dodge (1966) created a new group based on nuclear condition, termed the Mesocaryota, and included the dinoflagellates in this group. Subsequent work has,



however, tended to bring the dinoflagellates closer to the Eukaryota and has led to abandonment of the Mesocaryota.

According to Sigeo (1986), the dinoflagellates are most probably primitive rather than degenerate eukaryotic cells. The dinoflagellate chromosomes appear to be fundamentally different from those of all other eukaryotes.

Representative genera include *Ceratium*, *Gonyaulax*, *Gymnodinium*, *Peridinium*, and *Cryptothecodinium*.

### PHAEOPHYCEAE

These are virtually entirely marine and appear brownish. The largest known seaweeds, some exceeding many metres in length, belong to this class and these show a high level of anatomical differentiation.

Pigments are chlorophylls a and c, beta-carotene, fucoxanthin, and diatoxanthin. Laminarin and mannitol are the customary food reserves. Cells have one or more chloroplasts, often associated with pyrenoids. The chloroplast-pyrenoid system is covered by an ER envelope which is continuous with the nuclear membrane. Motile stages have two laterally-inserted unequal flagella.

Several brown algae produce sex hormones, and many show parthenogenetic development of gametes.

Elongated cells resembling sieve tubes occur in the medulla of Laminariales and some Fucales. In some of these kelps, e.g., *Fucus*, the epidermal (meristoderm) cells have their nucleus and plastids located basally (McCully, 1968). These cells have a hypertrophied perinuclear Golgi apparatus and a highly convoluted plasmalemma; most of the cell is occupied by membrane-bound vesicles which contain alginate, fucoidin, and certain phenolic substances.

### RHODOPHYCEAE

These red algae occur in both freshwater and marine habitats. They range from unicellular forms to filamentous, pseudoparenchymatous, and parenchymatous forms.

Their pigments are chlorophylls a and d, beta-carotene, phycoerythrin, and phycocyanin. Floridean starch is the chief food reserve. None of the red algae forms a flagellate stage. Sexual reproduction and life histories are quite complex and highly evolved.

The cell structure is basically simple. Cells have one or more simple chloroplasts, one nucleus and several Golgi bodies and mitochondria. Floridean starch is deposited not in the chloroplast but in the cytoplasm.

### CHLOROMONADOPHYCEAE (RAPHIDOPHYCEAE)

These unicellular flagellates are comparatively little studied. They lack cell walls and eyespots. They have large numbers of discoidal chloroplasts arranged in a single layer just below the cell membrane in *Gonyostomum*; in *Vacuolaria*, they occur in two or more layers. Pigments are chlorophylls a and c. The freshwater genera lack pyrenoids but the marine genus *Chattonella* has pyrenoids. The food reserve is oil, present in osmiophilic globules in the chloroplast stroma and elsewhere in the cytoplasm. A characteristic sheath of Golgi bodies surrounds the nucleus and a fibrous root connects the flagellar bases and the nucleus. The anterior flagellum has stiff hairs but the posterior one is smooth.

Besides chlorophylls, some carotenoids and xanthophylls may occur. The chloroplasts contain a single unbroken DNA ring in which the DNA is unevenly



distributed and exists as a microscopically-observable string of beads (Coleman and Heywood, 1981). Meiosis has not been reported in any species.

The systematic position and affinities of these algae are obscure. Three representative genera are named above. The new name of this class is Raphidophyceae (see Rosowski and Parker, 1982).

### GLAUCOPHYCEAE

Endocyanomes are associations between algae and blue-green endosymbionts (called cyanelles) and are placed in the class Glaucophyceae in a separate phylum Glaucophyta (Kies, 1980). Motile cells of this class are typically dorsiventral with two heterodynamic flagella. In *Glaucocystis* and *Gloeochaete*, the flagellar root system is ax cruciate with 4 multilayered structures. An elaborate pellicle consisting of a layer of flat vesicles (lacunae) lying just underneath the plasmalemma and associated with cytoplasmic microtubules is present. Starch accumulates in the cytoplasm. Cells have parabasal dictyosomes.

Mitosis and cytokinesis involve an open spindle, absence of centrioles, persistent spindle at telophase, and division of the cell by infurrowing of the plasmalemma. No phycoplast can be seen.

*Glaucocystis*, *Gloeochaete*, and *Cyanophora* are the three genera placed in this class.

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# 4 The Nucleus and Nuclear Division

## NUCLEAR ENVELOPE

Algal nuclei are typically bounded by a double-membraned envelope, each membrane being about 75 Å thick (Wischnitzer, 1973). Frequently, the outer nuclear envelopes evaginate to form ER. However, most of the ERs in the cytoplasm would have no connection with the nuclear envelopes; only a few have such connections. A space, around 20 nm wide, exists between the two membranes. The paired membranes are interrupted here and there by pores. A pore and the associated annular material together constitute the pore complex.

Nuclear pores appear to be constituted by fusions of inner and outer membranes which leave fenestrations of a mean internal diameter of 60–80 nm. The pore is encircled by eight symmetrically disposed, compact or loose granules, 10–20 nm each, which represent the structured components within the ring of annulus. The centre of the pore is often occupied by an electron opaque granule called central granule.

During mitosis, the nuclear envelope may persist (closed mitosis) or disperse (open mitosis). Three common patterns of the behaviour of the nuclear envelope during mitosis are: (1) complete dispersal at prophase, (2) complete persistence with no discontinuities other than the normal nuclear pores, and (3) the formation of polar fenestrae in an otherwise intact envelope (Fig. 4-1).

Examples of these patterns are now listed:

Dispersal of nuclear envelope at prophase: *Cryptomonas* sp., *Ochromonas danica*, *Chroomonas* sp., *Prymnesium parvum*, several diatoms, *Klebsormidium*, *Stichococcus*, *Coleochaete*, *Closterium*, *Cosmarium*, *Chara*.

Development of polar fenestrae: *Chlorella pyrenoidosa*, *Hydrodictyon reticulatum*, *Volvox aureus*, *Ulva mutabilis*, *Bryopsis hypnoides*, *Mougeotia* sp., *Polysiphonia* sp., *Zonaria farlowii*, *Pylaiella littoralis*.

Remains intact throughout mitosis: Euglenophyceae, dinoflagellates, *Vaucheria*, *Trentepohlia aurea*, *Stigeoclonium helveticum*, *Oedogonium*, *Bulbochaete*, *Acetabularia mediterranea*, *Pedinomonas minor*, *Griffithsia* sp.

Most algae exhibit two broad patterns of behaviour of the nuclear envelope at telophase. Type I is exemplified by *Astasia longa*, dinoflagellates, *Stigeoclonium helveticum*, and *Membranoptera platyphylla*. In these, after the chromosomes have moved to the poles, the nuclear envelope becomes medianly constricted. Type II is exemplified by *Euglena gracilis*, *Phacus longicaudus*, *Vaucheria litorea*, several green algae, *Porphyridium purpureum*, and *Polysiphonia* spp. In these, after the poleward migration of the chromosomes, the nuclear envelope is constricted in two places so that the interzone is cut off from the daughter nuclei.

Ueda *et al.* (1986) have reported a novel type of behaviour of the nuclear envelope in *Spirogyra* sp. In this species, the envelope becomes disrupted around the spindle equator at metaphase. Many small vesicles can be seen in the metaphase



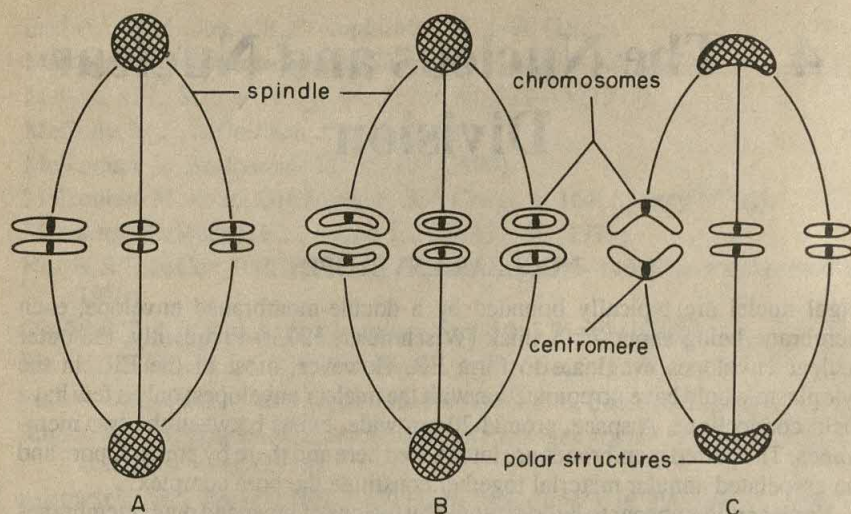


Fig. 4-1 Three patterns of behaviour of nuclear envelope during mitosis. A, the envelope disperses at prophase and spindle is open; B, the envelope disperses but forms individual envelopes, i.e., around each chromosome throughout mitosis (this mode is rare and occurs in the Sporozoan *Stylocephalus*); C, closed mitosis with intranuclear spindle. (After Heath, 1980.)

spindle. These vesicles surround the chromosomes and nucleolar material during early anaphase, and then fuse with one another to form the daughter nuclear envelopes. The interesting feature is that the nuclear envelope reforms around the chromosome groups during anaphase by fusion of small vesicles.

### NUCLEOLUS

One or more nucleoli of varying shape and size are formed within the nucleus during telophase. Unlike the nucleus, the nucleolus is not a membrane-bound structure. The nucleolus contains 16S and 25S ribosomes, and several chromosomes are intimately associated with the nucleolus. Some nucleoli are extrachromosomal.

The nucleoli are formed by certain specific regions of special chromosomes called nucleolar organizing chromosomes (Fig. 4-2). These are rather short segments of chromosomes, usually located in short arms of certain chromosomes. Nucleoli frequently contain certain inclusions which may be proteinaceous, carbohydrate, or lipid. They consist of at least three kinds of structural elements, viz., fibrillar, particulate, and nucleolar chromatin. The particulate zone seems to be located around the nucleolar surface and pervades those portions which are not occupied by fibrillar material. Commonly there is one nucleolar organizer per haploid chromosome set. The nucleolus organizer may be a special genetic locus containing a DNA template where active RNA synthesis can occur, resulting in the accumulation of an rRNA-containing product—the nucleolus.

During mitosis, the nucleoli disperse and degenerate at prophase, e.g., in Rhodophyceae, Phaeophyceae, Prasinophyceae, some desmids, *Bryopsis hypnoides*, *Coleochaete scutata*, *Trentepohlia aurea*, *Ulva*, *Klebsormidium*, *Chlamydomonas*, *Volvox*, Chlorococcales, diatoms, and Cryptophyceae. In *Euglena*, the nu-



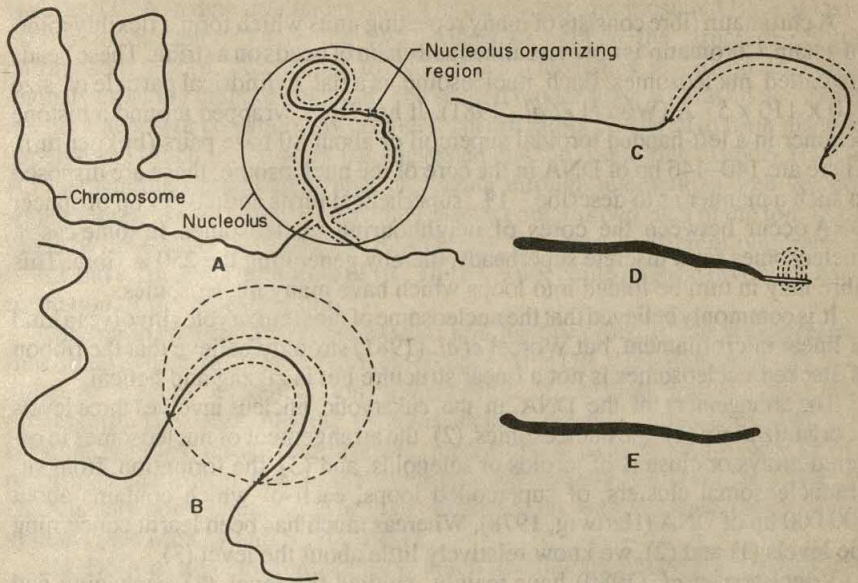


Fig. 4-2 *Spirogyra*, stages in nucleolar organization. A, coiled nucleolar organizing region of the chromosome within the nucleolus at interphase; B, uncoiling and contraction of nucleolar organizing chromosome at early prophase; C-D, dispersal of nucleolus at mid and late prophase; E, disappearance of nucleolus. (After Godward, 1966.)

cleolus persists or remains in its interphase form, becoming constricted into two equal halves at telophase. In *Cladophora*, the nucleolus fragments, and the fragments tend to persist around the nucleus. In *Spirogyra*, the nucleolus fragments but the fragments form a diffuse coating around the chromosomes.

In *Hymenomonas*, a narrow channel connects the nuclear envelope with the nucleolus (Riedmüller-Schömm, 1972).

In the vast majority of algae, however, nucleoli do not disperse or degenerate at prophase.

### NUCLEAR MATRIX

This is also called nucleoplasm. During mitosis, a small portion of the nuclear matrix is included in the daughter nuclei, whereas the remaining portion is discarded into the cytoplasm where it rapidly degenerates. In some algae, however, much of the matrix goes into the daughter nuclei.

### CHROMATIN

Chromatin of eukaryotic algae contains roughly equal proportions of histones and DNA. The finely-filamentous kind of chromatin that fills up much of the nucleus is called euchromatin, whereas the denser, granular chromatin is called heterochromatin. The euchromatic regions of a chromosome stain rather weakly with nuclear stains, whereas the heterochromatic regions stain deeply during interphase. The heterochromatic parts are thought to contain inactive genes.



A chromatin fibre consists of many repeating units which form a flexibly jointed chain. Chromatin is now visualized as a chain of beads on a string. These beads are called nucleosomes. Each nucleosome is a flat cylindrical particle of size  $110 \times 110 \times 57 \text{ \AA}$  (Worcel *et al.*, 1981). It has DNA wrapped around a histone octamer in a left-handed toroidal supercoil of about 80 base pairs (bp) per turn. There are 140–146 bp of DNA in the core of the nucleosome; these are disposed in such a manner as to describe  $1\frac{3}{4}$  superhelical turns and 20–95 bp of spacer DNA occur between the cores of neighbouring nucleosomes. In some cases, nucleosomes form discrete superbeads, thereby generating the 250 Å fibre. This fibre may in turn be folded into loops which have many nucleosomes.

It is commonly believed that the nucleosome of most eukaryotes involves a kind of linear microfilament, but Worcel *et al.* (1981) strongly believe that the ribbon of stacked nucleosomes is not a linear structure but is zigzag and helical.

The arrangement of the DNA in the eukaryotic nucleus involves three levels of organization: (1) the nucleosomes, (2) the arrangement of nucleosomes in ordered arrays or clusters of toroids or solenoids, and (3) the formation, from supranucleosomal clusters, of supercoiled loops, each of which contains about 100,000 bp of DNA (Hartwig, 1978). Whereas much has been learnt concerning the levels (1) and (2), we know relatively little about the level (3).

Vogelstein *et al.* (1980) have mainly studied the level (3) packaging and their results indicate that the nuclear DNA is organized in the form of supercoiled loops. When the nuclei are treated with non-ionic detergent and high salt concentration, their content of histones and other chromosomal proteins rapidly falls. From such depleted nuclei of interphase stage, intact loops of DNA are seen as a halo that surrounds a nuclear matrix or skeleton. By means of various enzymatic and labelling techniques, Vogelstein *et al.* were able to show that DNA replication occurs at fixed sites at the base of the loops and that the replicating DNA loops are motile with reference to their nuclear matrix attachment sites. An important implication of the latter observation concerning the motile behaviour of the supercoiled loops is that the DNA replication complexes are fixed and it is the loops which move through them as they are copied.

Chemical analyses of interphase chromatin from various eukaryotes have revealed three main levels of coiling: (1) the coiling of the DNA around the nucleosome, producing a 10-bp repeat, (2) the organization of histone into octamers, generating a 200-bp repeat, and (3) the restricted nucleolysis of chromatin, suggesting organization of nucleosomes into large domains, each having many nucleosomes. Burgoyne and Skinner (1981) have studied the level of organization between the nucleosome and the massive domains. They found that many nucleosomes exist in structures that cause every alternate nucleosome in a series to be resistant to attack by deoxyribonuclease.

Recent researches of Kornberg (1981) have established that the spacing of nucleosomes is not constant but variable (though not random) within a cell. In some cases, nucleosomes may be arranged in a regular as well as non-random manner. It is generally believed (Kornberg, 1981) that the arrangement of nucleosomes in chromatin is essentially random but is subject to two constraints, viz., (1) nucleosomes tend to be restricted to those regions of chromatin which have certain repressor or activator protein molecules and (2) nucleosomes contain about 166 bp of DNA each, and do not overlap one another. Kornberg feels that in many organisms the distribution of nucleosomes is mainly statistical rather than specific.

Researches during the past decade have shown that the chain of nucleosomes



is about 110 Å in diameter and is folded into a thicker chromosomal fibre of up to 300 Å in diameter. There are two models to explain the structure of this chromosomal fibre. According to one, the nucleosomes are disposed continuously as a long helix or solenoid. The second model suggests that nucleosomes are not linearly or continuously arranged but are rather clustered to form discrete superbeads. Much recent work has suggested that the lysine-rich histones H1 and H5 mediate the supernucleosomal packaging through interactions with nucleosomes (Jorcano *et al.*, 1980); each nucleosome contains two each of the four main kinds of histones comprising an (F2A1)<sub>2</sub>(F3)<sub>2</sub> tetramer, a different oligomer of F2A2 and F2B, and a monomer of F1. The F1 is not believed to be involved in the formation of the repeating unit.

Chromatin material also contains some non-histone proteins. The structural and functional dynamism of the DNA is chiefly due to the DNA-binding proteins which affect or mediate its replication, transcription, translation, transposition, and processing. The DNA-binding proteins include the polymerases, synthetases, twisting proteins, untwisting proteins, repressors, activators, and histones. The precise mechanism of how these various proteins actually interact or bind with the DNA is only now beginning to be understood, but no work has been done on algal systems (see McKay and Steitz, 1981).

The DNA of algal chromosomes is much like that of other eukaryotes. Some of the cytosines are methylated. The resulting 5-methylcytosine residues occur in specific sequences, usually adjacent to guanine residues at the 3'-end of the DNA molecule. In some cases and for certain genes, methylation of DNA sequences is associated with transcription inhibition. In *Chlamydomonas*, selective degradation of paternal chromosomes is thought to be caused by a lack of DNA modification at certain sequences (see Ehrlich and Wong, 1981). Methylation of cytosine in the plastid DNA of female gametes of *Chlamydomonas* seems to protect this DNA against inactivation.

Methylated cytosine residues constitute preferred sites for spontaneous mutation. Enrichment of certain sequences in 5-methylcytosine is an important factor determining the higher order structure of chromosomes. This view is based on the following evidences: (1) non-random distribution of 5-methylcytosine residues along the surface of metaphase chromosomes and all through chromatin and (2) relative abundance of 5-methylcytosine residues in satellite DNAs and in repetitive DNA sequences. 5-methylcytosine plays an important role in the organization of polytene chromosomes. DNA methylation is also involved in the repair of damaged DNA and in differentiation. According to Ehrlich and Wong, the multifold uses of DNA methylation may include: (1) gene transcription regulation, (2) rise in mutation rates, (3) increase in the stability of the DNA double helix, (4) alteration in the conformation of DNA, chromatin, and/or chromosomes, and (5) occasional prevention of nucleolytic degradation.

Unlike most other classes of algae having normal chromatin, the Euglenophyceae interphase nuclei have distinct chromosomes evenly distributed in the nuclear matrix (Ueda, 1960). The matrix is more granular and less dense than typical matrices of other classes. The most characteristic feature of euglenoids is that their chromosomes are much more highly condensed than in most other eukaryotes (Leedale, 1967). Another very significant cytological observation is the demonstration of localized centromeres on the chromosomes of *Euglena* during mitosis (Gillott and Triemer, 1978).

Lefort-Tran and associates have studied the nuclear condition in cells of



*Euglena gracilis* that had been starved of vitamin B<sub>12</sub>. During starvation, the nucleus enlarged in the same proportion as the cell itself (Bre *et al.*, 1980, 1982). Whereas in normal cells the chromatin was always condensed (Fig. 4-3), in starved cells it was completely dispersed. While the nucleosomal organization was preserved (Fig. 4-4), the chromatin of the starved cells was more sensitive to nuclease digestion.

Bre and Lefort-Tran (1978) also examined the influence of vitamin B<sub>12</sub> starvation on the cortex of *Euglena*. This cortex is quite complex and displays a highly repetitive pattern of ridges and grooves; it replicates at each division by intussusceptive growth (Fig. 4-5). Vitamin B<sub>12</sub> starvation blocks this process and makes the cell surface increase exclusively by elongation of the parental ridges (Bre and Lefort-Tran, 1978).

Bre *et al.* (1981) showed that certain specific proteins must be synthesized for an orderly progression of mitosis to occur in *Euglena*.

The chromosomes of the Dinophyceae are also quite different from those of other algae. They are highly condensed (Figs. 4-6, 4-7), transversely banded (Dodge, 1966), and lack histones. They contain the unusual base hydroxymethyluracil (Rae, 1976), but lack nucleosomes (Herzog and Soyer, 1981). Some dinoflagellates undergo an unusual kind of mitosis in which the nuclear envelope remains intact but many cytoplasmic channels containing microtubules run completely through the nucleus (Oakley and Dodge, 1974). The chromosomes remain attached to the nuclear envelope but during division this attachment assumes the form of a narrow stalk of chromosomal material that fits into a depression in the nuclear membrane lining the cytoplasmic channels. Oakley and Dodge (1974) describe the presence of definite kinetochores in *Amphidinium carterae*; these connect together the channel microtubules and the nuclear envelope immediately adjacent to the positions of attachment of chromosomes on the nuclear side of the envelope. Thus, contrary to earlier reports, the dinoflagellates do seem to have a spindle. This mitotic spindle is entirely cytoplasmic although it does pass through the nucleus in special channels (Oakley and Dodge, 1977).

Some striking features of nuclear division in dinoflagellates include: (1) chromosome condensation and persistence of nuclear envelope throughout the nuclear division cycle, (2) absence of typical eukaryotic metaphase plate, (3) arch-shaped chromosomes similar to bacterial nucleoids and their attachment to the nuclear membrane during chromosomal segregation (Oakley and Dodge, 1974), (4) absence of centriole and of centromeric heterochromatin (Haapala and Soyer, 1974), (5) presence of a permanently extranuclear spindle (Kubai and Ris, 1969), and (6) absence of nucleosomes in the chromatin of *Prorocentrum micans* (Herzog and Soyer, 1981) and of *Cryptothecodinium cohnii* and *Peridinium balticum* (Rizzo and Burghardt, 1980).

Certain cryptophytes also show non-typical mitosis. A study of *Chroomonas salina* by Oakley and Dodge (1973) has brought to light the following features: (1) flagellar bases divide and microtubules extend from these to the cytoplasm just around the nucleus; (2) nuclear envelope breaks down and permits the microtubules to extend into the nucleus; (3) at metaphase, the chromatin material aggregates into a dense plate at the equatorial region; many spindle microtubules penetrate and cross through the chromatin mass and the equatorial plate; (4) at anaphase, two dense clumps of chromatin separate and neither discrete chromosomes nor kinetochores or centrioles can be seen during or before anaphase; and (5) at telophase, the chromatin clumps are pushed back into the endoplasmic





Fig. 4-3 Electron micrograph through section of *Euglena gracilis* Z, showing a mitotic nucleus. Microtubules occur in several planes, one microtubule being seen parallel to a stretched nucleolus. The formation of new cortical ridges during karyokinesis can be seen. mt, microtubules; nr, new cortical ridges. (x12,900.) Courtesy M. Lefort-Tran.



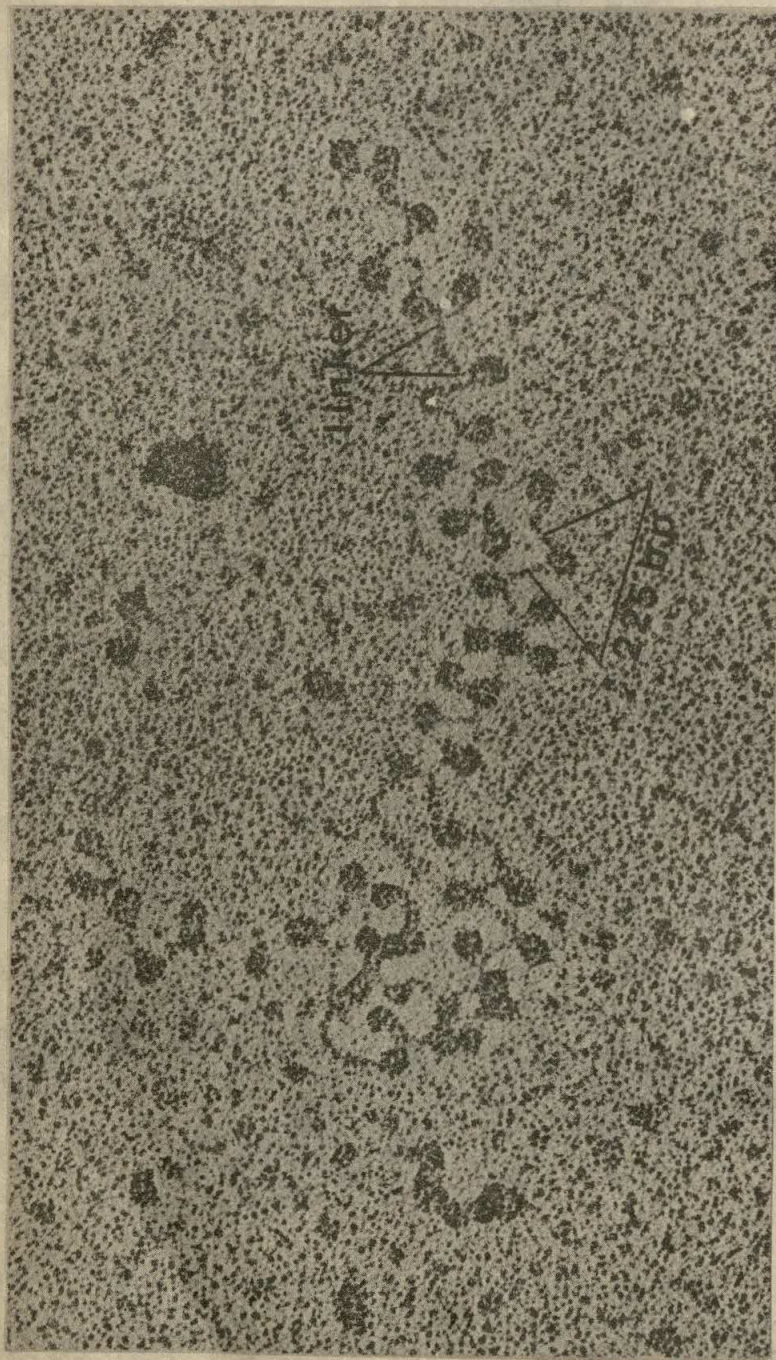


Fig. 4-4 Electron micrograph of chromatin of *Euglena* (spread according to the technique of Dubochet), showing helical nucleosomal structure. In this chromatin, the DNA repeat length is 225 base pairs and the DNA linker is 59 bp. (x200,000.) Courtesy M. Lefort-Tran.



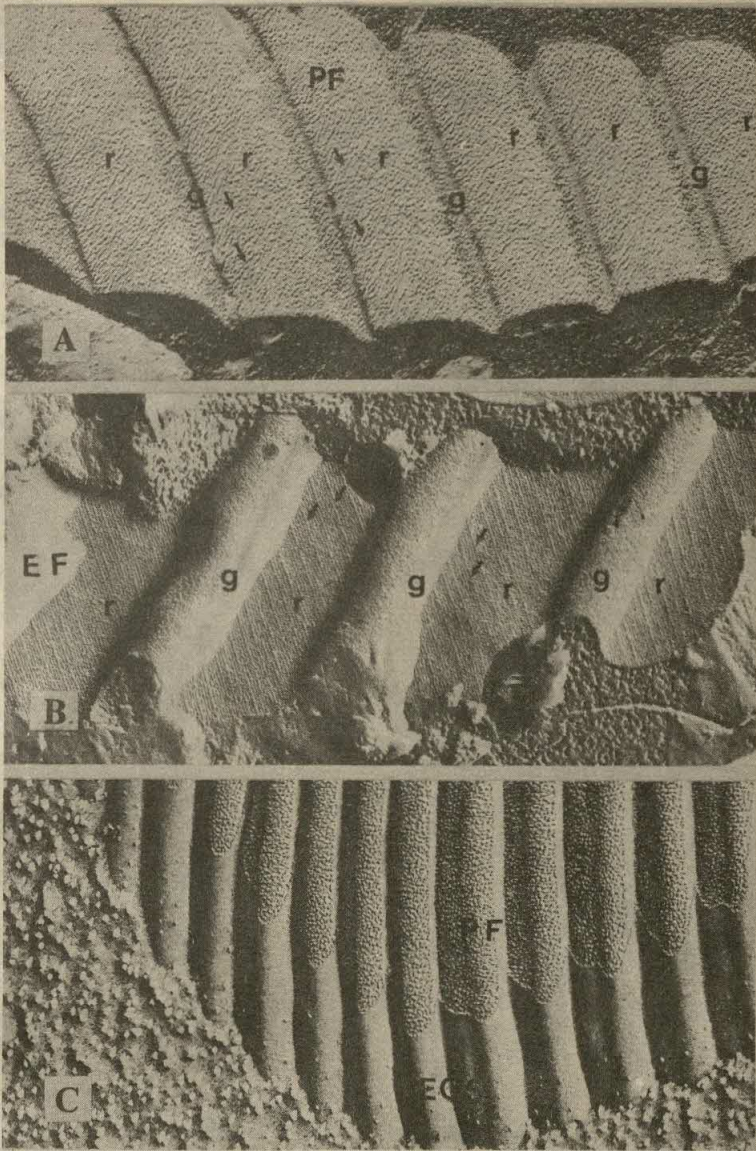


Fig. 4-5 Freeze fracture electron micrograph of pellicle of *E. gracilis* Z. A, protoplasmic fracture face with particles. Ridges and grooves alternate and the grooves are free from particles. Some particles on ridges are close together and form rows or striations (arrows) with the same major orientation as that on complementary fracture face. PF, protoplasmic fracture face; r, ridges; g, grooves. (x35,500.) B, exoplasmic fracture face. The striated structure of the ridges appears fairly well in contrast to the convex surface of the grooves devoid of such organization. The regular striations are at an angle of about  $40^\circ$  to the direction of the strips and are separated from one another by 6.3 nm. More apparent striations can be seen with a periodicity of about 40 nm (arrows). EF, exoplasmic fracture face. (x59,100.) C, Freeze fracture with etching, showing the true smooth outer surface of the pellicle, over the particulate protoplasmic fracture face. EOS, smooth outer surface of pellicle; PF, protoplasmic fracture face. (x39,400.) Courtesy M. Lefort-Tran.



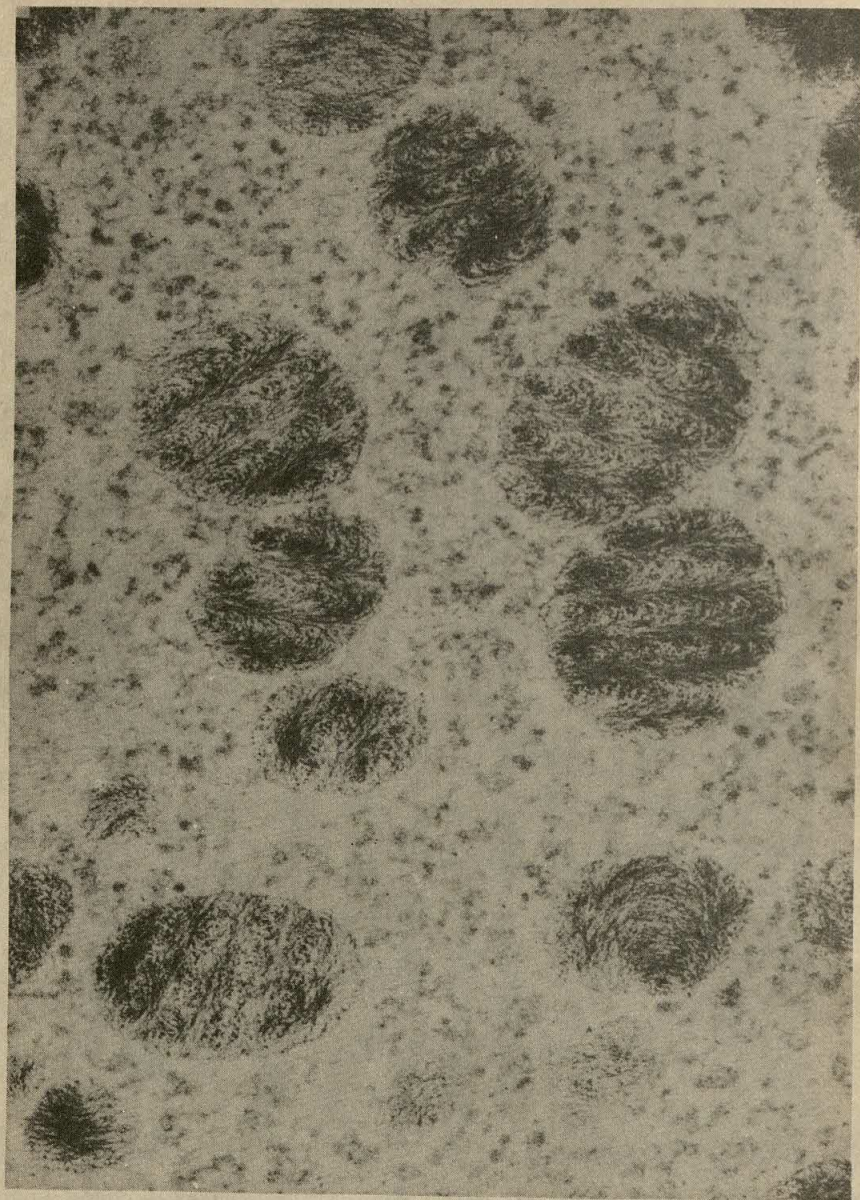


Fig. 4-6 Electron micrograph of chromosomes in a resting nucleus of *Peridinium bipes*. (x57,000.) Courtesy K. Ueda.

reticulum sheath which surrounds the chloroplasts; this seems to form the first part of nuclear envelope, with additional envelope material being synthesized on the opposite side of the nucleus.

#### PERINUCLEAR RETICULUM

Some Chlorococcales and *Porphyridium* develop a cisternum of mostly non-



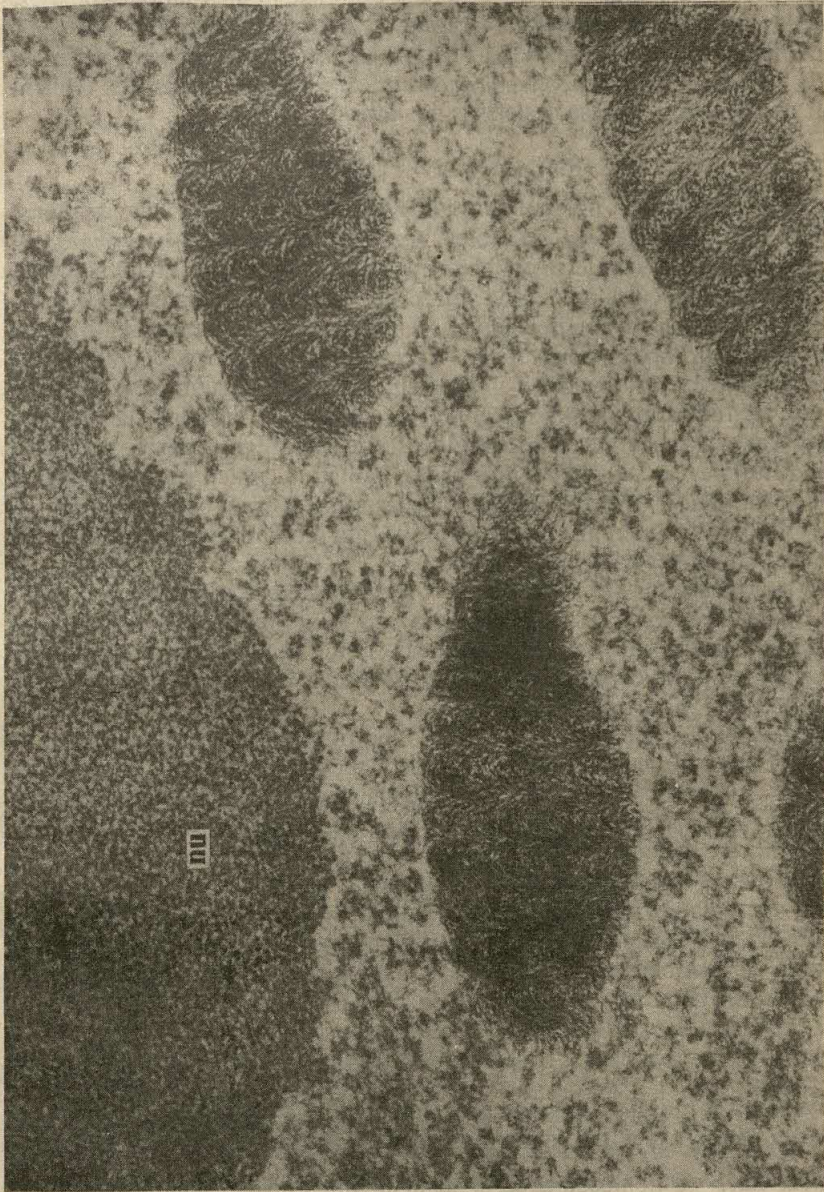


Fig. 4-7 *Amphidinium cryophilum*. Electron micrograph of a portion of nucleus showing a granular nucleolus and dinokaryotic chromosomes. nu, nucleolus. (X22,800.) Courtesy L. W. Wilcox.



fenestrated endoplasmic reticulum around their mitotic nuclei (Heath, 1980).

### VESICLES

Some algae contain large vesicles or tubular cisternae in their mitotic spindles. Examples are *Ochromonas danica*, *Prymnesium parvum*, *Vacuolaria virescens*, several diatoms, *Ulothrix fimbriata*, *Klebsormidium*, *Spirogyra*, some desmids, *Nitella*, *Chara*, *Griffithsia*, *Membranoptera*, and *Polysiphonia* spp. (Heath, 1980). The function of these vesicles is not known.

### POLAR STRUCTURES

These structures lie at the poles of the mitotic spindle and exhibit great variations. Heath (1980) designates as 'centrioles' those structures which are obviously related to the array of 9 triplet microtubules and their associated linking structures; he uses the term 'nucleus-associated organelles' (NAO) for the range of all other polar structures. In many euglenoids, basal bodies lie close to the spindle poles, but to one side. In *Prymnesium parvum*, *Vaucheria litorea*, *Chlorella pyrenoidosa*, *Hydrodictyon*, several other green algae and brown algae, the centrioles lie at right angles to each other. In *Kirchneriella* and *Tetraedron* which lack flagellated stages but still have centrioles, the centrioles move to the polar regions during prophase. These centrioles are involved in spindle formation which at this stage is extranuclear. *Chlorella* also lacks flagellated stages but forms centrioles (Wilson *et al.*, 1973). In the interphase, these centrioles are present in a depression in the nuclear envelope nearest to the cell wall.

No polar structures are known in the Cryptophyceae, Conjugales, and some other algae. *Oedogonium* lacks centrioles and forms an entirely intranuclear spindle. Its nuclear envelope remains intact during mitosis. It forms complex kinetochores on the chromatids during prophase. Somewhat simpler kinetochores occur in *Spirogyra* and *Cladophora*.

In *Oedogonium*, the spindle is closed. Fine filaments (up to about 8 nm in diameter) are attached to kinetochores from prophase through metaphase to anaphase. Some are free in the early division nucleus, whereas others arise from developing kinetochores at prophase at a stage when there are very few microtubules within the nucleus. At prometaphase, microtubules penetrate the nucleus and in some manner interact with the microfilaments. At this stage, or even earlier, several microtubules associate laterally with kinetochores. As the mitosis progresses, the microfilaments are interspersed among the microtubules of kinetochore fibres (Schibler and Pickett-Heaps, 1980). These workers have demonstrated, for the first time, fine filaments attached to the kinetochores of *Oedogonium*. They have also studied their disposition in the spindle from prophase through anaphase. The mitotic stages observed in *Oedogonium* are the following:

**Prophase** The nucleus elongates, becoming spindle-shaped. The chromosomes condense and pack together within the nuclear wall. Nucleolus starts to disperse.

**Prometaphase** Chromosomes show some jiggling movement at the two poles, become displaced centrally, clearing the polar regions of the nucleus. Nucleus enlarges. Chromosomes spread outward from the centre and also exhibit some rolling movements. They become attached at their slightly constricted centromere regions to spindle fibres. Then they migrate toward the equatorial plane. The nucleus assumes a spherical shape.



**Metaphase** The kinetochore pairs align across the equator. Paired chromatids start splitting longitudinally.

**Anaphase** Chromosomes migrate polewards. Nucleus again elongates thereby separating the chromosomes still farther apart. Then the chromosomes decondense and form the new daughter nuclei. The elongated spindle collapses and the nuclei flatten against one another. A phycoplast is organized and cytokinesis occurs (Schibler and Pickett-Heaps, 1980).

In *Hydrodictyon reticulatum*, the nuclei of vegetative cells divide semi-synchronously. Mitosis begins with the replication of the centrioles, the emergence of microtubules between the centrioles, and the establishment of the spindle poles. The nucleolus disperses at prophase, and an extranuclear sheath of microtubules is formed and is enclosed by the perinuclear envelope. Microtubules enter into the nucleus through pores in the nuclear wall and organize into an intranuclear spindle. The spindle elongates and the reforming nuclear envelope at anaphase isolates the interzonal apparatus from the daughter nuclei.

In *Microspora*, the perforated nuclear envelope persists through nuclear division and centrioles are present at the poles of the semi-open spindle.

An unusually interesting feature observed in *Klebsormidium* by Pickett-Heaps (1975) is the constancy of the chromosome-to-pole distance during mitosis.

*Vaucheria* has a closed, centric spindle lacking polar fenestrae, with the centriole situated outside the nuclear envelope at the poles (Ott and Brown, 1972). This type of spindle is quite unusual for green algae (*Vaucheria* is a yellow-green Xanthophyceean alga that was formerly placed in the Chlorophyceae) but occurs in some phycomycetous fungi.

*Ochromonas danica* has rhizoplasts. Diatoms form striate bars. Small ring-shaped polar structures have been recorded in *Polysiphonia* spp. (Heath, 1980).

In *Porphyridium*, the NAO is a complex, bipartite granule, whereas in *Polysiphonia* it is a short, hollow cylinder termed a 'polar ring' (Scott *et al.*, 1981). In *Polysiphonia* and *Dasya*, polar rings can be observed during all mitotic stages and may even be present in reproductive branches (Scott *et al.*, 1981). Especially in *P. harveyi*, polar rings are persistent organelles, and they may be permanent organelles in other red algae as well. In fungi and diatoms, nuclear associated polar structures are involved in the assembly and organization of the ordered arrays of microtubules which constitute the major part of the spindle apparatus. In *P. harveyi*, longitudinally-oriented microtubules surround the nucleus, but it is not yet established whether the associated polar rings directly influence the formation or spatial organization of microtubules.

## SPINDLE

This is the central and the most important component of mitosis. The term "spindle" means the spindle-shaped metaphase nucleus itself; "spindle fibre" is very different from "spindle". Microtubules constitute the primary components of spindles. The pole-to-pole framework of the spindle can be either intranuclear or extranuclear. It is composed mainly of either truly pole-to-pole, continuous microtubules, or of interdigitating microtubules originating from opposite poles. The framework may either be coherently bundled centrally around whose periphery the chromatin is disposed, or it may be quite dispersed, forming a sort of array in which the chromosomes lie scattered.



The chromatin material interacts with the spindle via kinetochores and kinetochore microtubules that intermingle with or run along with the framework microtubules. The number of microtubules per kinetochore can vary from one (*Heteromastix*, *Porphyridium*, *Cryptomonas*) to two or more (*Tetraspora*, *Euglena gracilis*) to many (*Nitzschia*, *Hydrodictyon reticulatum*, *Oedogonium*, *Bulbochaete*, *Spirogyra*). *Oedogonium* characteristically forms large, multilayered, complex kinetochores. During metaphase, these kinetochores and chromosomes align at the metaphase plate and spindle tubules become attached to them. At anaphase, the kinetochores split up and migrate polewards, the rest of the chromatid trailing behind. The kinetochores become connected to the polar regions by means of microtubules. Then the nucleus elongates and interzonal microtubules appear between the groups of chromatids. Later, the nuclear envelope contracts around the groups of daughter chromosomes, isolating them from the spindle which soon collapses.

Algae exhibit two common modes of spindle formation. In Mode A, the polar structures may develop small arrays of only kinetochore microtubules prior to their separation, but the bulk of the spindle is formed as the polar structures migrate apart. Examples: most diatoms, *Blastodinium*, and *Syndinium* (dinoflagellates). In Mode B, the polar structures migrate to the distance apart that they will occupy at metaphase; it is only after this migration that the spindle develops. Examples: *Phacus longicaudus*, *Euglena gracilis*, *Ochromonas danica*, *Vaucheria litorea*, and most green and red algae.

Diatoms are especially valuable for studying mitosis. According to Pickett-Heaps *et al.* (1982), the kinetochores initially interact with microtubules nucleated at or emanating from the poles, during normal mitosis in diatoms. They postulate that the kinetochore is best studied as an organelle capable of generating motility. Diatoms also have uniquely well-ordered spindles. The microtubules comprising a diatom spindle consist of two equivalent interdigitated half-spindles, with the microtubules comprising each half-spindle having uniform polarity. Some of the microtubules in each half-spindle interact during prophase, thus creating a parallel set of microtubules between the poles, the "central spindle". The remaining microtubules splay outwards from the poles and constitute the structural basis for chromosome movements and subsequent attachment to the central spindle (Pickett-Heaps *et al.*, 1982). The kinetochores do not significantly nucleate microtubules; rather, they move along microtubules emanating from the pole. According to Pickett-Heaps *et al.*, the kinetochore is a key kinetic organelle in mitosis, not just the anchoring point of an active traction fibre. It may well be an energy transducer that can generate tension.

By metaphase, an electron dense "collar" is formed around the microtubules, and extends between the kinetochore and the poles. Pickett-Heaps *et al.* (1982) believe that this collar is a part of the spindle's traction apparatus. During anaphase, the half-spindles actively slide apart. At telophase, the half-spindles disassemble, starting from the non-polar end and proceeding back to the poles.

Particular emphasis has been placed by Pickett-Heaps *et al.* (1982) on the function of the kinetochore in diatoms. Kinetochores are believed to function primarily by interacting with, and sliding along, microtubules from the poles. At prometaphase, one kinetochore moves towards either pole, whereas the second moves away from that pole. Prometaphase chromosome movement to the pole is not exactly the same as that which occurs away from the pole; kinetochores may therefore respond to the intrinsic polarity of the microtubules. In these algae, the



chromatin enables the kinetochores to stretch rather close to the poles during metaphase. At the time of chromosome splitting at anaphase, the kinetochores complete their poleward movement. In this movement, the collar material seems to play some role as it extends between the poles and the kinetochores and contracts polewards during anaphase.

Light microscopic observations on red algal mitoses have suggested that the nuclear envelope breaks down during mitosis, but this conclusion is not supported by recent ultrastructural studies which have revealed that open spindles are absent in these algae and that except for polar fenestrae in *Porphyridium*, *Membranoptera*, and a few other genera, the nuclear envelope remains intact during mitosis (Bronchart and Demoulin, 1977). *Porphyridium* is especially unusual in having a single large microbody at each spindle pole.

The presence of polar fenestrae indicates that red algal mitosis is not completely intranuclear. In *Membranoptera*, a distinctive group of chromosomes-to-poles microtubules are attached by structured kinetochores; such attachment is very uncommon in plants (Heath, 1974).

The spindle in the Cryptophyceae is generally barrel-shaped, wider than long, and located near the anterior end of the cell with its axis being perpendicular to the long axis of the cell. During spindle formation, microtubules reorientate through 90° angle so as to align themselves in parallel with the spindle. The flagellar bases and associated material appear to act as microtubule nucleating centres but do not have any role in ordering the microtubules into a parallel array (Oakley, 1978). In *Cryptomonas* sp. and *Chroomonas salina*, during interphase a few microtubules extend from the flagellar base pair at the anterior end of the cell to the posteriorly located nucleus. Prophase begins with the replication of the flagellar bases and the proliferation of the associated microtubules. The microtubules extend from the amorphous material surrounding the bases and from various flagellar roots. The numbers of microtubules increase as the nucleus migrates toward the cell anterior and the replicated flagellar base pairs migrate apart, finally coming to lie anterior to and on either side of the nucleus. The nuclear wall then breaks down and most of the microtubules enter the nucleoplasm. The microtubules may at first extend in all directions, or may dispose themselves as per the final spindle alignment pattern from the beginning (Oakley, 1978). All these events point to the fact that the process of mitosis in the Cryptophyceae is patently unique.

Greenwood (1974) discovered a small, double-membraned body, called the nucleomorph, in the periplastidal compartment of cryptomonads. The periplastidal compartment is the space between the chloroplast ER and the chloroplast envelope; in this space, there occur a number of distinctive inclusions, this being a unique feature of the cryptomonads. The inclusions may be starch grains, scattered tubules and vesicles, and ribosomes, and the most distinctive inclusion is the nucleomorph (Fig. 4-8). According to Greenwood *et al.* (1977), the nucleomorph represents the vestigial nucleus of an ancestral eukaryotic endosymbiont. According to Ludwig and Gibbs (1985), this endosymbiont was an ancestral red alga. Ludwig and Gibbs have shown the presence of DNA in the nucleomorph of *Cryptomonas abbreviata*.

The spindle-equivalent (i.e., the microtubules) in the dinoflagellates is entirely cytoplasmic and in this respect contrasts with most other organisms, except euglenoids, where it is partly cytoplasmic and partly nuclear (see Dodge, 1973); in euglenoids, it is entirely intranuclear.

Cells of all dinoflagellates have a nucleus (called the dinocaryotic nucleus) with



permanently condensed chromosomes and quite rich in its P, Ca, Fe, Ni, Cu, and

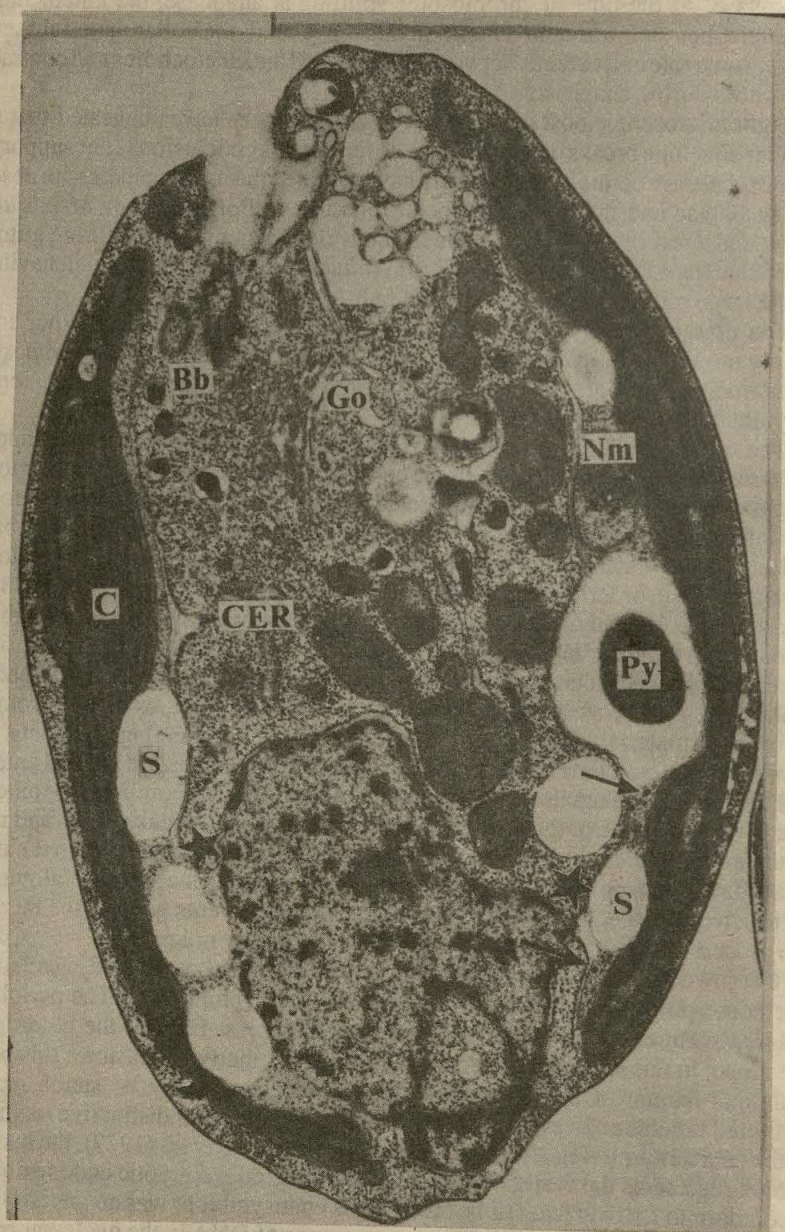


Fig. 4-8 *Cryptomonas* sp. Longitudinal section through an early preprophase cell showing the presence of starch grains, ribosomes, dense globules, and a nucleomorph in the periplastidal compartment. Bb, basal bodies; C, chloroplast; S, starch; arrows, dense globules; Nm, nucleomorph; CER, chloroplast ER; stars, nuclear envelope; Go, Golgi; Py, pyrenoid. (x18,800.) (After McKerracher and Gibbs, 1982.) Courtesy S.P. Gibbs.



Zn contents. Besides this dinocaryotic nucleus, another nucleus (called the supernumerary nucleus) is found in *Peridinium balticum* and *Glenodinium foliaceum*; this supernumerary nucleus completely lacks Fe and Ni and has permanently dispersed chromatin, thus resembling the interphase nucleus of eukaryotic cells (Sigeo and Kearns, 1981).

Gillott and Gibbs (1980) studied the nucleomorph ultrastructure and observed that its double membrane is interrupted by pores and larger gaps. During cell division, chloroplast division and nucleomorph division precede mitosis, the nucleomorph remaining within its periplastidal compartment. Occasionally, the nucleomorph's outer membrane is continuous with the tubules of the periplastidal compartment (Fig. 4-9). The nucleomorph divides in preprophase (Fig. 4-8)

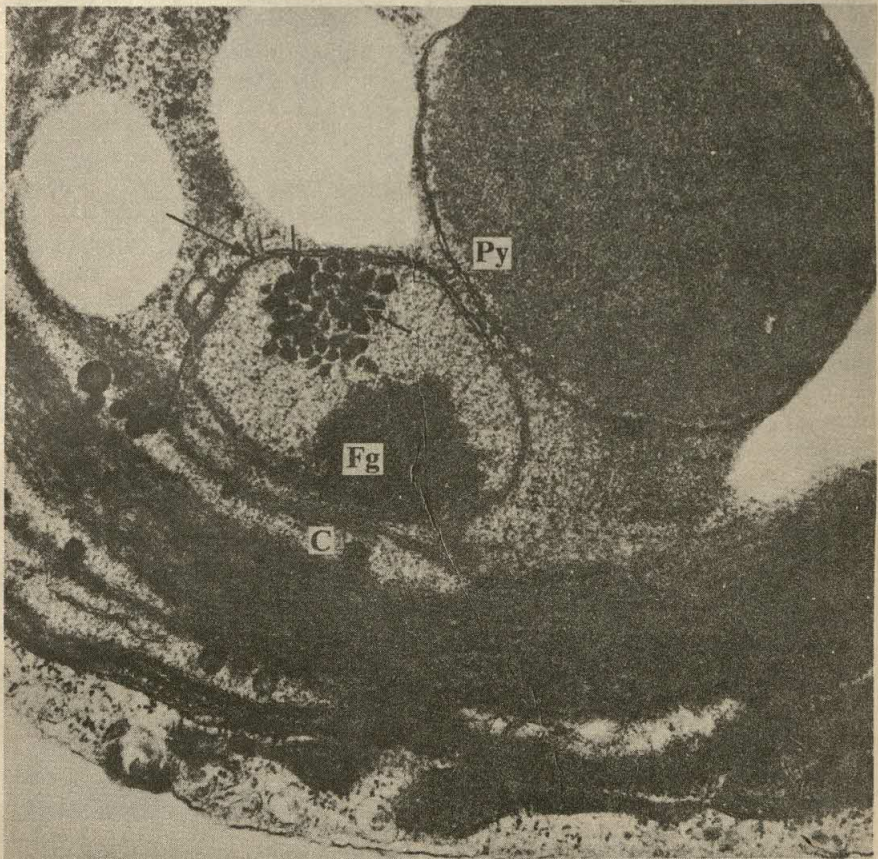


Fig. 4-9 Nucleomorph of *Cryptomonas* sp. The nucleomorph envelope is continuous with periplastidal tubules at the large arrows. The fibrillogranular body lies adjacent to the chloroplast. Fg, fibrillogranular body; Py, pyrenoid; C, chloroplast. (x39,000.) (After Gillott and Gibbs, 1980.) Courtesy S.P. Gibbs.

following basal body replication but before division of the chloroplast and its chloroplast endoplasmic reticulum is completed (McKerracher and Gibbs, 1982). The inner membrane of the nucleomorph envelope invaginates first



forming a double-membraned baffle. Then the outer membrane invaginates, completing the division. Microtubules are not involved in the nucleomorph division.

Nucleomorphs contain two distinctive inclusions, viz., dense globules and dense fibrillogranular body (Fig. 4-10). The latter contains nucleic acid (Gillott and Gibbs, 1980).



Fig. 4-10 *Cryptomonas* sp. Dividing nucleomorph. A central double-membraned baffle has formed by the invagination of the inner membrane of the nucleomorph envelope. Globules and a fibrillogranular body are present in each half. Fg, fibrillogranular body; G, globules. (x55,250.) (After McKerracher and Gibbs, 1982.) Courtesy S.P. Gibbs.

The location of the nucleomorph within a cell varies from species to species. In *Chroomonas salina*, *Cryptomonas reticulata*, *C. maculata*, and *Rhodomonas lens*, it occurs in an invagination of the pyrenoid. In *Chilomonas paramecium*, several species of *Chroomonas* and *Cryptomonas*, and *Hemiselmis salina*, it occurs



along the inner surface of the chloroplast. Except in dividing cells, there is only one nucleomorph per cell.

According to Gillott and Gibbs (1980), the nucleus-like features of the cryptomonad nucleomorph support the hypothesis that cryptomonads have descended from a symbiotic association between two eukaryotic organisms.

The important characteristic of dinoflagellates is that they have permanently condensed chromosomes throughout their cell cycle. The work of Spector *et al.* (1981) suggests that the dinoflagellate chromosome contains a single chromonema composed of helically arranged DNA strands. The unwound chromosome has two important properties, viz., (1) symmetry along its main axis and (2) a typical change of aspect between cross sections and longitudinal sections.

The Prasinophyceae are heterogeneous in so far as the types of mitosis and cytokinesis are concerned. Thus, *Pedinomonas*, *Pyramimonas*, and *Nephroselmis* show a persistent spindle similar to that observed in the class Charophyceae; a collapsing mitotic apparatus and phycoplast resembling those of the Chlorophyceae are encountered in the genus *Platymonas* (Mattox and Stewart, 1977). The mitotic mechanism and cytokinesis of *Mantoniella squamata* have been described by Barlow and Cattolico (1981). Before the onset of mitosis, the reniform cells enlarge and become globose. The chloroplast, the eyespot, the pyrenoid, the Golgi body, the mitochondrion, and the basal bodies all replicate or start to divide during the preprophase stage and their duplication is completed by or during early prophase. The daughter flagella move apart. Numerous microtubules appear in the cytoplasm and then enter the nuclear area via polar fenestrae. The nuclear envelope remains intact and persists throughout division, but the nucleolus disperses. In metaphase, as the chromosomes migrate further apart, the spindle pole-to-pole distance increases in keeping with the alignment of chromosomes at the metaphase plate. At this stage, the basal bodies lie laterally adjacent to the poles. The single microbody, which is V-shaped, elongates and extends into depressions in the pyrenoid stalk. At anaphase, the cell becomes markedly bilobed because of the separation of the daughter chloroplasts from each other. The chromosome-to-pole distance decreases progressively. As the dividing cell progresses into telophase, the division of the microbody is completed, the nuclear region becomes pronouncedly bilobed, and the cleavage furrow incises the cell. This cleavage furrow is actually derived from the coalescence of vesicles from the Golgi apparatus and the endoplasmic reticulum. The vesicles which originate from the Golgi body have spiderweb scales. Both nuclear division and cytokinesis are completed at the same time (Barlow and Cattolico, 1981).

The findings of Barlow and Cattolico and those of previous workers on the Chlorophyceae and Prasinophyceae provide support for the views of Pickett-Heaps and Ott (1974) and Stewart and Mattox (1975) that such Chlorophycean algae as *Trichosarcina* and *Pseudendoctonidium* may have evolved from an asymmetrical, naked or scaly flagellate, e.g., *Pedinomonas*, and not from a *Chlamydomonas*-like ancestor.

A unique type of mitosis has been reported in the chrysophyte *Ochromonas danica* (Slankis and Gibbs, 1972). During interphase, the basal bodies of the two flagella replicate and the chloroplast constricts between its two lobes. The rhizoplast, which is a fibrous striated root attached to the basal body of the long flagellum, runs under the Golgi body along the surface of the nucleus in interphase cells. The unique feature is that the two rhizoplasts form the poles of the spindle, with the spindle microtubules inserting directly on to the rhizoplasts. Whereas



some microtubules of the spindle extend from pole to pole, others seem to attach to the chromosomes.

The cytological studies of algal nuclei and mitoses have given valuable clues to the phylogenetic relations and evolutionary status of various algal phyla. These studies have dispelled the common belief that the Cryptophyceae, Chrysophyceae, Haptophyceae, and Chloromonadophyceae are particularly primitive. The nuclear and mitotic characters of these classes are fairly advanced in many ways. Cytologically, the dinoflagellates and euglenoids seem to be much less advanced evolutionarily as compared to the remaining algal classes.

### CHROMOSOME NUMBERS

The haploid number of chromosomes in the Volvocales varies from  $n = 4$  (*Astrephomene gubernaculifer*, *Chlamydomonas monoica*) to  $38 \pm 4$  in *C. eugametos* (Sarma, 1982). In the Chlorococcales, the range is  $n = 4$  (*Scenedesmus platydiscus*, *S. arcuatus*) to  $n = 80$  in *Eremosphaera viridis*. In the Ulotrichales,  $n = 3$  in *Prasiola japonica*, whereas  $n = 48$  in *Hormidium crenulatum*. In some taxa of this order, chromosomal races exist. *Draparnaldiopsis indica* (Chaetophorales) has  $n = 4$ , whereas *Trentepohlia uncinata* has  $n = 56$ . Euploid series with  $2n$  ranging from 12 to 144 seems to exist in some members of the Cladophorales, where the basic number is  $n = 6$ . Some species of *Cladophora* and *Rhizoclonium* show polyploidy. In the Oedogoniales,  $n$  ranges from 9 to 46.

Some species of *Spirogyra* and certain desmids have polycentric chromosomes. Some Japanese *Spirogyras* have  $n = 2$  which seems to be the lowest recorded chromosome number for any alga (Tatuno and Iiyama, 1971). In contrast, *Netrium digitus* has  $n = 592$  (King, 1960). Among the siphonous green algae, *Bryopsis plumosa* has the lowest number with  $n = 4$ , whereas the highest ( $n = 20$ ) occurs in *Codium decorticatum*.

Among the green algae, aneuploidy has played substantial role in the evolution of different taxa belonging to the Volvocales, Chlorococcales, Chaetophorales, Oedogoniales, and Conjugales (see Sarma, 1982).

In the Charales, the lowest number of chromosomes recorded is  $n = 6$  (*Nitella* spp.) and the highest ( $n = 70$ ) in *Chara zeylanica*. Intraspecific polyploidy prevails in several taxa of the Charales, and some instances of aneuploidy have also been recorded.

In the Euglenophyceae,  $n = 4$  in *Euglena gracilis*, and  $n = 182(\pm)5$  in *E. spirogyra* var. *fusca*.

Precise chromosomal counts have not been made in the case of the Dinophyceae but some species, e.g., *Endodinium chattonii*, may have over 1000 small chromosomes! In some dinoflagellates, however, the chromosomes are fairly long and rod-like.

The remaining classes of algae do not seem to have been adequately investigated from the viewpoint of their chromosome numbers (Sarma, 1982).

### MEIOSIS

Some reports on the ultrastructural aspects of meiosis in algae include the work of Manton *et al.* (1969, 1970) on the marine centric diatom *Lithodesmium*, Bräten and Nordby's (1973) work on *Ulva*, Kugrens and West's (1972) study on *Janczewskia* and other red algae, and Triemer and Brown's (1976) investigation in *Chlamydomonas reinhardtii*. Of course, several workers have studied algal meiosis under the light microscope.



*Lithodesmium* has a spindle precursor made of a series of parallel plates. Mitotic spindle consists of microtubules whose number increases rapidly with the progress of prophase. In meiosis, the number of microtubules increases during the first division but in the second division it is reduced to one-half and the spindle is somewhat smaller. The number of microtubules at Meiosis-I ranges from about 165 to 190, that at Meiosis-II from 82–140, and that at mitosis about 100–105. In all three cases, the microtubules occur in 16 bundles.

In *Ulva*, the series of events involved in meiosis was found to be generally identical to that in mitosis. This alga, like many red algae and *Chlamydomonas*, forms distinct synaptonemal complexes. Synaptonemal complexes usually appear in meiotic chromosomes during late zygotene, each unsynapsed chromosome forms a longitudinal proteinaceous thread-like structure; two such threads (one each from a homologous chromosome) then align parallel to each other, constituting the two lateral elements of a synaptonemal complex. These lateral elements

become connected by strands that extend from the externally situated chromosomes, through the lateral elements, to form a central element lying in between the two lateral elements. All the three elements are extrachromosomal and are lost after meiosis. These structures are responsible for synapsis, defined as the configuration of paired homologues. The synaptonemal complexes are not always correlated with effective cross-overs and chiasmata. The synaptonemal complex (Fig. 4-11) seems to hold homologous chromosomes intimately together right

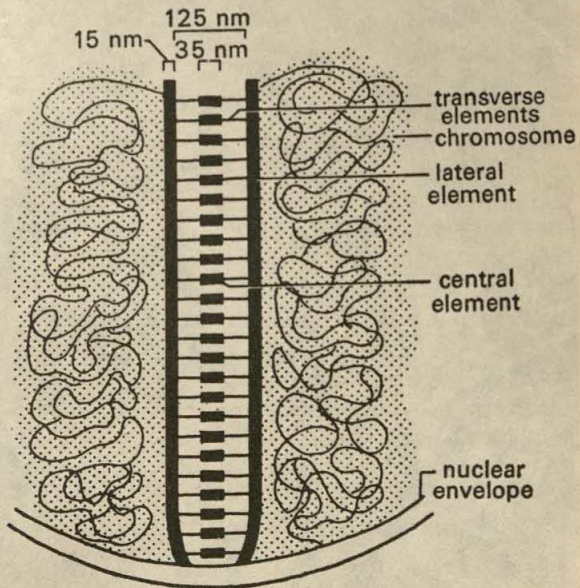


Fig. 4-11 Diagrammatic sketch to illustrate the structure of a synaptonemal complex.

through pachytene. Its disintegration at diplotene leads to the loosening apart of the homologous chromosomes except at chiasmata; at chiasmata, some remnants of the complex may still remain. Brown (1972) has proposed that the period from the commencement of synthesis of synaptonemal complex to the end of pachytene, when the complex is discarded, be called synaptotene.

Kugrens and West (1972) have recorded synaptonemal complexes in sections of young tetraspore mother cells of *Janczewskia gardneri*, passing through the nucleus (Figs. 4-12, 4-13).

The ultrastructure of meiosis in germinating zygotes of *Chlamydomonas reinhardtii* has been studied by Triemer and Brown (1976). The interphase nucleus is densely granular, with a conspicuous nucleolus. Basal bodies reappear at late



interphase or early leptotene. Axial cores appear at leptotene when the chromo-

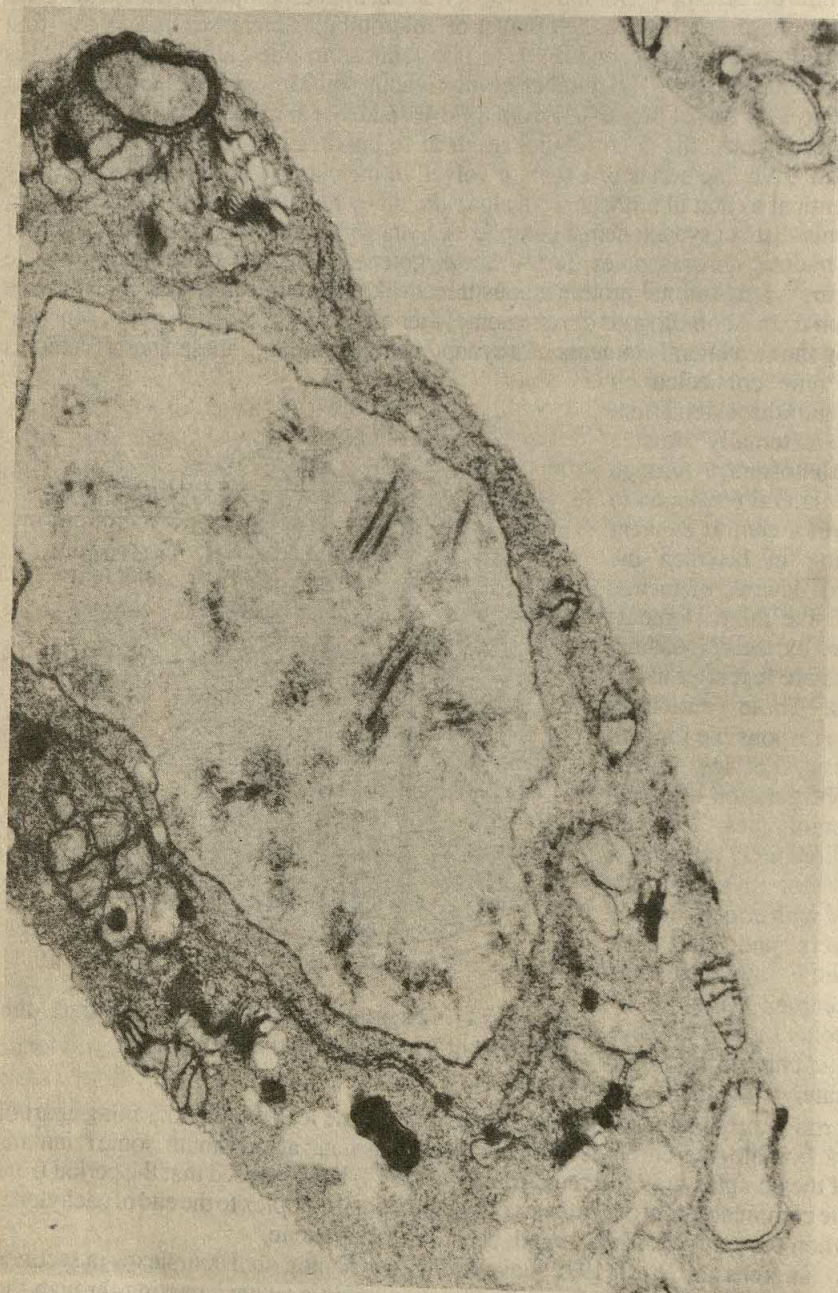


Fig. 4-12 Electron micrograph of section through tetraspore mother cell of the red alga *Janczewskia gardneri*, showing several synaptonemal complexes in the nucleus. In the cytoplasm, several mitochondria, proplastids, Golgi bodies, and endoplasmic reticulum are seen. (x17,450.) Courtesy P. Kugrens.





Fig. 4-13 Electron micrograph through longitudinal section of a synaptonemal complex of *J. gardneri*, showing a possible attachment to the nuclear membrane (bottom). (x104,750.) Courtesy P. Kugrens.



nemata condense. By zygotene, axial elements associate with the nuclear envelope, certain tubules are formed in the perinuclear space adjacent to the associated chromosomes, and bivalents associate with the nucleolus. Synaptonemal complexes are evident during pachytene when chromosomes are still attached to the nuclear wall, whereas the tubules are no longer seen in the perinuclear space. The chromosomes are highly condensed at diplotene-diakinesis, and the synaptonemal complexes degenerate. Chiasmata are observable at this time but not the nucleolus. By Metaphase-I, the nuclear envelope disintegrates at the poles, a spindle is formed, and spindle microtubules attach to the chromosomes. In this alga, the nuclear envelope remains intact throughout meiosis (also through mitosis) except at the nuclear poles. Daughter nuclei re-form at telophase. By Metaphase-II, the basal bodies migrate to the poles. The completion of second meiotic division results in the production of haploid zoospores which later grow into *Chlamydomonas* individuals.

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# 5 The Cytoplasm and Subcellular Organelles

## CYTOPLASM

High-voltage electron microscopes have been employed to observe the fine structure of cells. With these microscopes, a very high degree of optical resolution is achieved. Earlier it used to be believed that the cell cytoplasm was an indistinct, structureless medium in which membrane-bound organelles such as mitochondria, ribosomes, endoplasmic reticulum, and Golgi bodies are suspended. During the last decade, it has been found that the cell has a dynamic and elaborate network of skeletal elements comprising of three different systems, viz., microfilaments, microtubules, and intermediate filaments.

With the recent development and installation of high-voltage electron microscopes in some American laboratories, the ground substance of the cell cytoplasm has been carefully examined and this has revealed a system of gossamer filaments that support and move the cell organelles; this system is called "the microtrabecular lattice" (Porter and Tucker, 1981). The structure of this lattice varies in response to changes in cell shape or in the cellular environment. The microtrabecular lattice partitions the cell into two phases: (1) the polymerized, protein-rich phase of the lattice itself and (2) the fluid, water-rich phase which fills the interstices within the lattice.

The lattice is thought to play some role in organizing the enzymes in the cytoplasm, and also in cell differentiation and the associated protein synthesis; recent observations have shown that the polysomes do not just float around randomly in the cytoplasm but rather lie at the intersections of the lattice in a neat and regular pattern.

The microtrabecular lattice extends through the entire cell. It seems to connect together the disparate components of the cytoplasm (viz., the structural fibres and the subcellular organelles) into a single unit of structure and function called the "cytoplast". The lattice also mediates regulated and directed transport within the cell. The control of cell's shape and movement appears to depend on the integrated functioning of the microtubules, microfilaments, microtubule-organizing centres, and the microtrabecular lattice (Porter and Tucker, 1981).

Most eukaryotic cells exhibit some uniform, continuous transport of organelles and cytoplasm along fairly regular pathways. A good example is the rotational streaming of endoplasm in many algal cells. Another kind of intracellular motility observed in many algae involves discontinuous, erratic movements of particles and organelles (Schliwa, 1984). The first type of movement (cytoplasmic streaming) is widely believed to be associated with microfilaments, and the second type with microtubules. Analogous phenomena in animal cells include muscle contraction (associated with microfilaments) and ciliary movement (based on microtubules). This comparison has further prompted some biologists (see Schliwa, 1984) to suggest that microfilament-mediated cytoplasmic transport may be an actomyosin-based contractile process, whereas microtubule-associated



transport might probably involve a dynein-like ATPase (see also Chapter 7).

Microfilaments made of actin (F-actin) occur in the cytoskeleton of many protists. These filaments are usually 6 nm in diameter and 1 or more  $\mu\text{m}$  long. They may occur singly or in bundles or networks. They may be located at the periphery of the cell or be intracellular, or in the contractile ring of division. In *Synura*, short microfilaments are stretched between the membrane of the scale-forming vesicles and that of the chloroplast along which the vesicles are displaced (Mignot and Brugerolle, 1982). These microfilaments are responsible for the anchorage of the scale-forming vesicles along the chloroplast; they form ribbons of superimposed filaments which vanish after the scales have mineralized.

Actin is present in the cytoplasm, but not the cortex, of the euglenoid *Distigma*. However, this actin does not occur in the form of microfilaments. It plays an important role in the euglenoid movement.

Protists also contain certain non-actin microfilaments. Some of these non-actin filaments are elements of contractile systems whereas others are not. No such non-actin filaments are known to occur in metazoans.

Certain fibrous structures are associated with kinetosomes in protists. Kinetosomal periodic rootlets are composed of tightly arranged fine filaments, bearing regularly arranged thickened areas or bands. These rootlets are quite widespread in flagellates; *Chilomonas* has roughly cylindrical or blade-shaped rootlets. The rhizoplasts of *Tetraselmis* are attached at their deep end, one to the nuclear membrane and the other to the plasma membrane. These rhizoplasts are abnormally long (over 400 nm) and are contractile (Grain, 1986).

The giant internodal cells of *Chara* and *Nitella* have been the systems of choice for various studies of cytoplasmic streaming. In these cells, streaming is restricted to a rather narrow zone between the stationary, chloroplast-containing ectoplasm and the large central vacuole. The streaming endoplasm describes a spiral path along the long axis of the cell, turns around near the end, then streaming back at the opposite side of the cell. A variety of cytoplasmic inclusions move along with the endoplasm. The studies of Nagai and Rebhun (1966), Pickett-Heaps (1967), and Palevitz and Hepler (1975) demonstrated the presence of microfilaments and actin, and pointed to the involvement of actomyosin in the streaming process. It seems highly probable that streaming in such cases is generated by actin-myosin interactions (Sheetz and Spudich, 1983).

Kamiya (1959) studied the movement of chloroplasts and nuclei in the stalk of *Acetabularia* and observed it to proceed in numerous channels in a thin layer of cytoplasm along the cell wall. Nagai and Fukui (1981) showed that streaming often occurred in opposite directions in different channels at the same time.

Microtubules play a role in the streaming of cytoplasm in *Caulerpa* and other algae. Dawes and Barilotti (1969) showed that in *Caulerpa*, bundles of cytoplasmic microtubules are oriented parallel to the axis of the endoplasmic streams. Sometime, chloroplasts occur in close proximity to these bundles, aligned along their long axes. *Caulerpa* seems to lack microfilaments.

## MICROTUBULES

Microtubules constitute important multipurpose morphogenetic tools in eukaryotic cells. They regulate the cell shape of algal cells, and specify the site and plane of cell division. Spindle microtubules play an indispensable role in mitosis. One interesting point is that microtubules do not function as individuals so much as they do in the form of microtubule-arrays or aggregates.



Tubulin is the major structural unit of microtubules. In *Chlamydomonas*, it accounts for some 5% of the total protein synthesized over much of the cell cycle.

Plant cytoplasmic microtubules consist of 13 protofilaments, each protofilament being a linear chain of alternating alpha- and beta-tubulin. All 13 protofilaments are aligned in the same way, slightly staggered so that the dimers lie in helices around the microtubular wall (Gunning and Hardham, 1982). This means that microtubules are polar structures.

Microtubules constitute extremely important elements of the cytoskeleton. They are involved in flagellar locomotion, shape maintenance, cytokinesis, nuclear division, migrations and positioning of intracellular organelles. The simplest microtubular systems are made of only 1 or 2 microtubules and are present in some ciliates. Somewhat less simple systems are strips or ribbons of microtubules joined to each other in a single plane; a good example of this category is the ribbon of *Chilomonas* which remains associated with a dense lamella or with periodic lamellae over its entire length (Fig. 5-1).

This alga also has tangential microtubule systems which occur in the form of ribbons or radiating sets of microtubules which leave the base of the kinetosome laterally and run beneath the cell pellicle (Fig. 5-1).

Cytoplasmic microtubules play an important role in the aggregation of zoospores of *Pediastrum*, *Hydrodictyon*, and *Sorastrum* to form the characteristic colony shape (Marchant and Pickett-Heaps, 1974; Millington and Gawlik, 1975). Many ovoid algal cells and zoospores, such as those of *Frittschiella*, contain cytoskeletal arrays of microtubules in their peripheral cytoplasm (Melkonian, 1975).

Microtubules mediate organelle movements within cells. In *Closterium*, postmitotic nuclear movement occurs along a groove between two recently-divided chloroplasts. A microtubule-centre appears near the nucleus and migrates along the path which is later taken by the postmitotic nucleus. Microtubules run back from the centre toward the nucleus, one point extending along the trail of microtubules and the remaining bulk of the nucleus being dragged behind it. A distance of about 20  $\mu\text{m}$  is travelled in 7–15 min (Pickett-Heaps and Fowke, 1970). Similar nuclear migrations also occur in *Micrasterias*.

### CHROMATOPHORES (CHLOROPLASTS)

Most eukaryotic algal cells have one or more chromatophores whose internal structure is fairly constant for a particular class but varies from class to class (Table V).

Most chromatophores are limited by double membranes with a space of 70–100 Å between the two layers. This is so in the Chlorophyceae, Prasinophyceae, and Rhodophyceae. A three-membraned envelope is characteristic of the chromatophores of the Euglenophyceae (Figs. 5-2, 5-3) and Dinophyceae. However, *Amphidinium*, *Woloszynskia*, and a few other dinoflagellates have chloroplasts with only two bounding membranes (Wilcox *et al.*, 1982). In the remaining classes, the envelope is four-membraned. In green algal chloroplasts, the outer membrane is quite smooth (Fig. 5-4). However, in chloroplasts of *Chlamydomonas* (Fig. 5-5), the outer membrane contains patches of particles here and there (Lefort-Tran, 1982). The outer membrane has a low protein/lipid ratio and a non-specific permeability to low molecular weight metabolites. The inner membrane of green algal chloroplasts differs from the outer membrane in showing much selective permeability for certain anions and in the synthesis of carotenoids, galactolipids, etc.



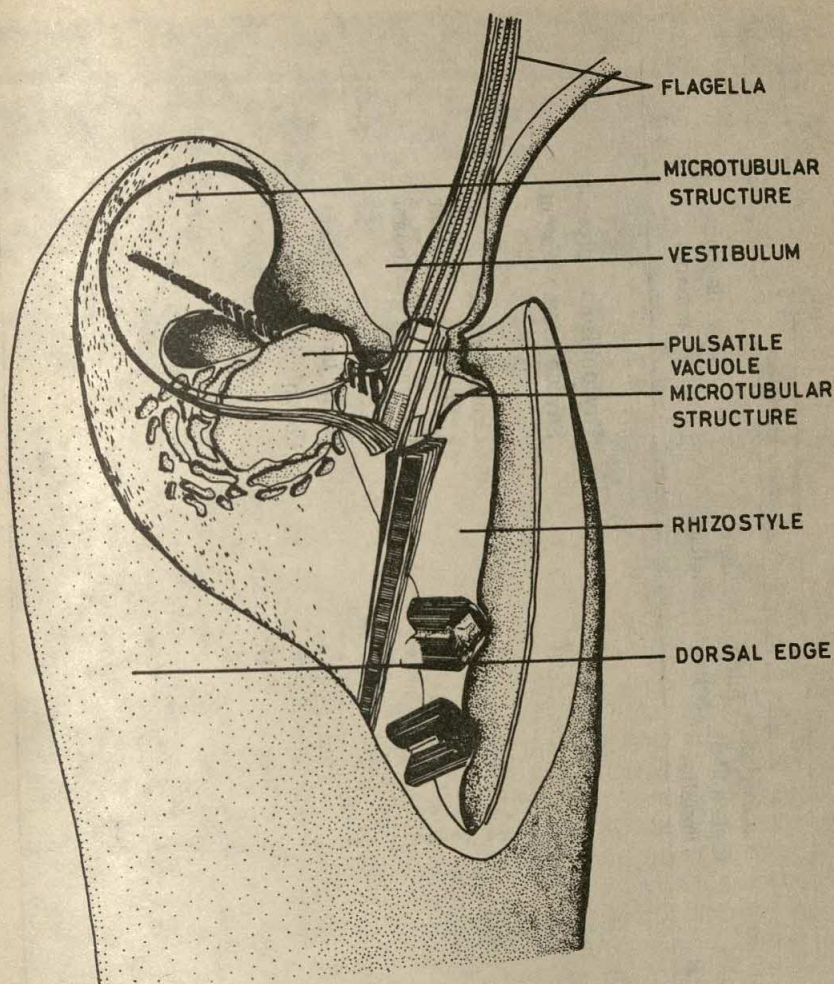


Fig. 5-1 Diagrammatic sketch of 3-dimensional reconstruction of the anterior part of *Chilomonas* showing the microtubular structures, rhizostyle, vestibulum, and flagella (after Mignot *et al.*, 1968).

(Lefort-Tran, 1981).

Lefort (1962) made use of certain mutants of *Chlorella* to study the role of photosynthetic pigments on the structure of the chloroplasts. Mutants lacking the ability to synthesize chlorophyll in the dark were used to analyze the correlation between the blockage of carotenoid and chlorophyll synthesis and chloroplast differentiation (Fig. 5-4). Figure 5-4 compares the fine structure of chloroplast in the mutant 5/520 of *Chlorella vulgaris* grown in the dark with that grown in the light.

The chloroplast of *Euglena* has a three-layered envelope. The outermost membrane may be ergastoplasmic in nature, or it may be vacuolar, or may be equivalent to a plasma membrane. The density of particles along this membrane is different from that in the other two membranes (Lefort-Tran, 1981). The polar distribution between the two complementary fracture faces is such that the



Table V Main characteristics of chloroplast structure in algae

Class	Plastid envelope		Number of thylakoids/ lamella	Plastid lamellae	
	Number of membranes around plastid	Connections with ER		Interconnections between lamellae	Miscellaneous remarks
PROKARYOTIC					
Cyanophyceae	No discrete plastid		1	-	Phycobilisomes present
Prochlorophyceae	No discrete plastid		2	?	Phycobilisomes absent
EUKARYOTIC					
Chlorophyceae	2	-	2 to many	+	Grana formed
Tribophyceae	4	+	3	+	Girdle lamella present
Bacillariophyceae	4	+	3	+	Presence of girdle lamella uncertain
Phaeophyceae	4	+	3	+	Girdle lamella present
Rhodophyceae	2	-	1	-	Phycobilisomes present
Euglenophyceae	3	-	3	Uncertain	
Chrysophyceae	4	+	3	+	
Dinophyceae	3	-	3	-	
Eustigmatophyceae	4	±	3	-	
Prasinophyceae	2	-	2-4	+	
Cryptophyceae	4	+	2	-	
Haptophyceae	4	+	3	+	



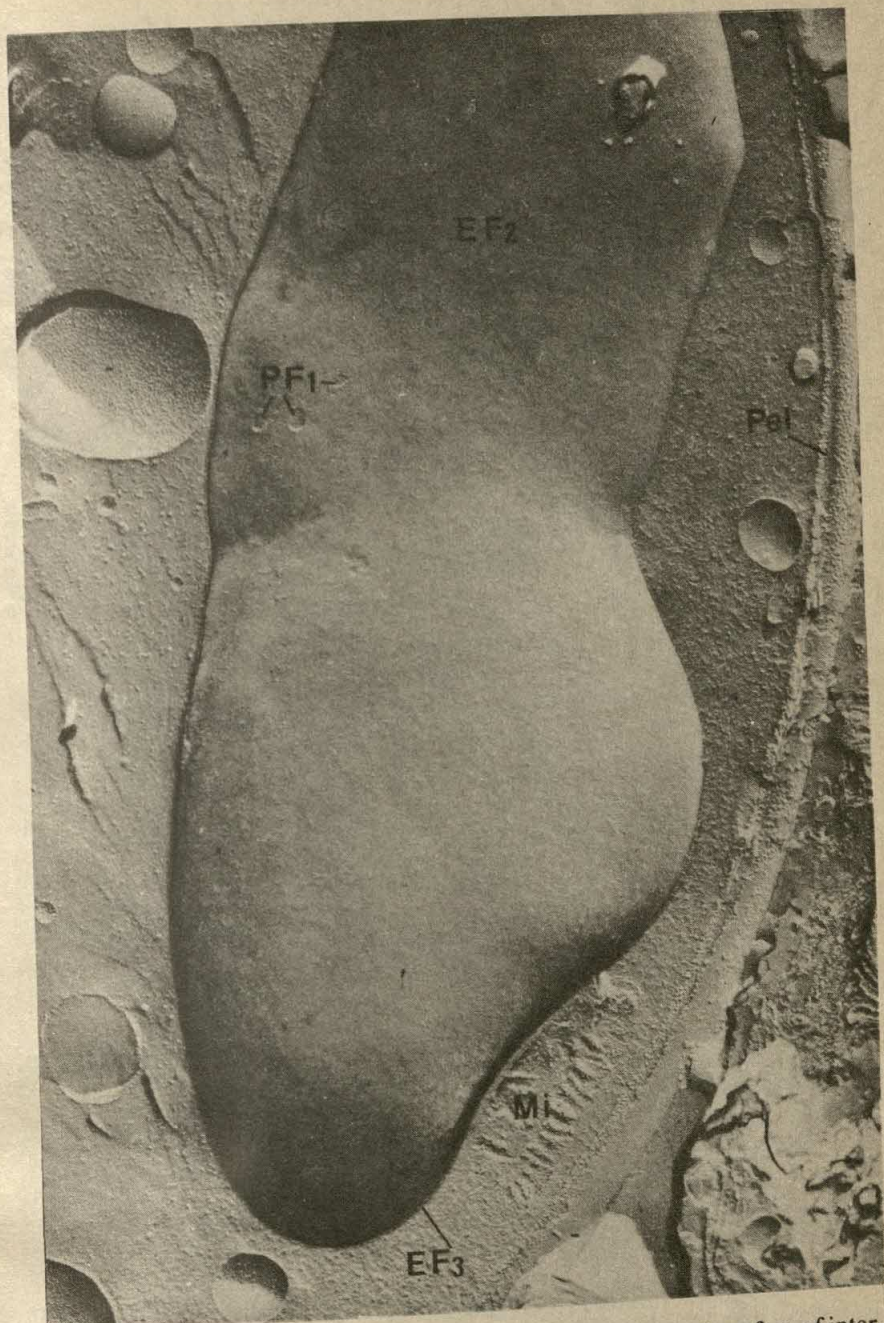


Fig. 5-2 *Euglena* chloroplast *in situ*. The convex EF<sub>2</sub> smooth surface of intermediary chloroplast membrane with only windows shows the PF<sub>1</sub> fracture face of the inner membrane of the envelope. The perichloroplastic surface of the envelope is seen as a rim EF<sub>3</sub>. Pel, pellicle; Mi, mitochondrion. (x27,000.) Courtesy M. Lefort-Tran.



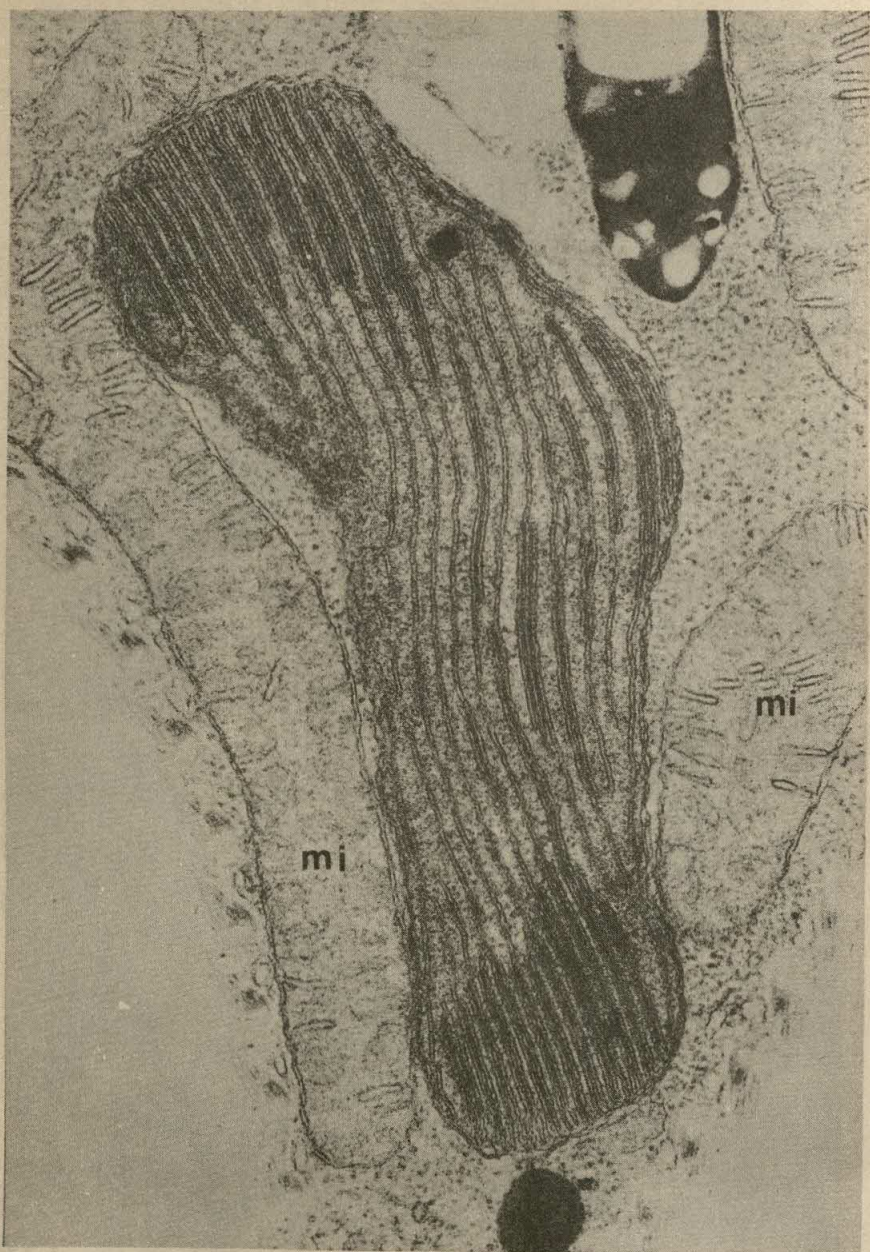


Fig. 5-3 *Euglena* chloroplast greened under light flashes. Thylakoids are isolated or appressed but without true partitions. The triple layered envelope can be seen. Mi, mitochondrion. (x42,750.) Courtesy M. Lefort-Tran.

enriched concave fracture PF face is toward the cytoplasm, whereas the convex face, which has very few particles, is exoplasmic.

According to Lefort-Tran (1981, 1982), the euglenoids may have evolved from



eukaryotic hosts into which green algal chloroplasts became endosymbiotic. The perichloroplastic envelope (the outer, third membrane) would then be homologous

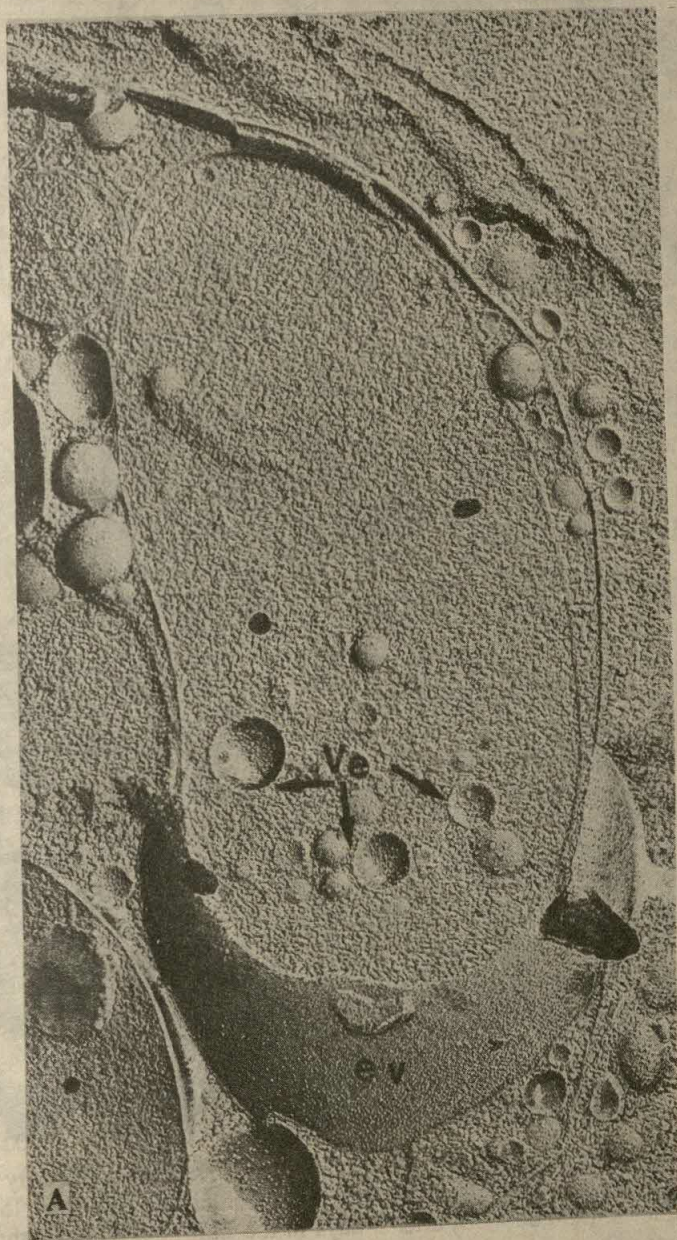


Fig. 5-4 *Chlorella vulgaris*, mutant strain 5/520. A, in the dark only the envelope and a few vesicles in the matrix can be observed. ev, envelope; Ve, vesicles. (x50,000.) B, after 5 days in the light, the photosynthetic membranes have grown; typical grana form from two to ten thylakoids. Fracture face EF with a few particles and PF with many particles, can be seen. thy, thylakoids; g, grana. (x50,000.) Courtesy M. Lefort-Tran.





Fig. 5-4 *Chlorella vulgaris*, mutant strain 5/520. A, in the dark only the envelope and a few vesicles in the matrix can be observed. ev, envelope; Ve, vesicles. (x50,000.) B, after 5 days in the light, the photosynthetic membranes have grown; typical grana form from two to ten thylakoids. Fracture face EF with a few particles and PF with many particles, can be seen. thy, thylakoids; g, grana. (x50,000.) Courtesy M. Lefort-Tran.

with the vacuolar membrane of the host cell. Lefort-Tran is of the opinion that the chloroplasts of red and green algae have originated from cyanobacterial endo-



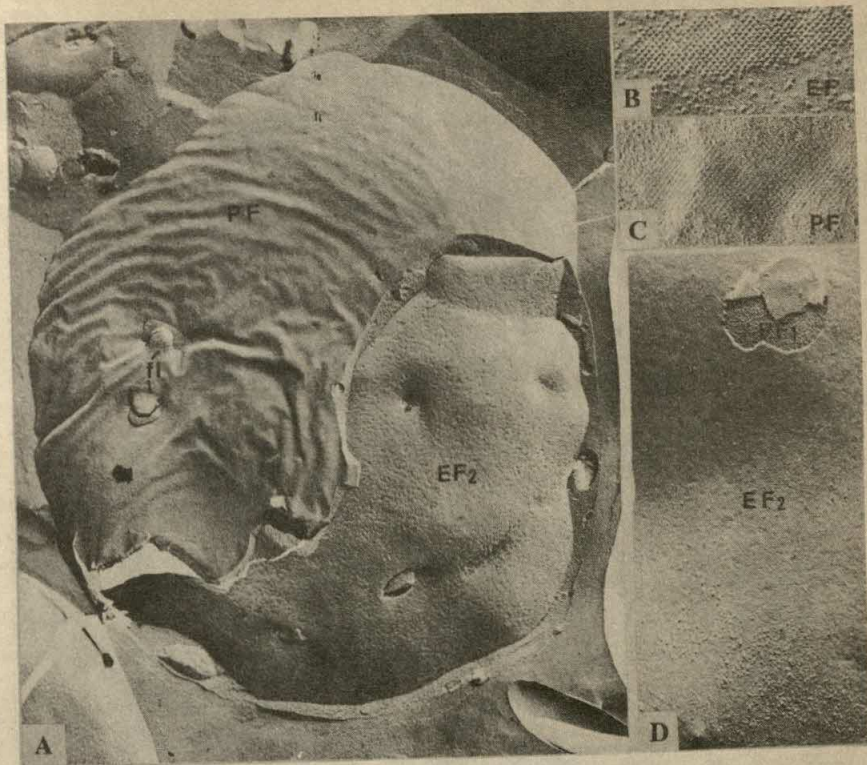


Fig. 5-5 *Chlamydomonas stellata*. A, convex protoplasmic PF fracture face of the plasma membrane bearing prints of organized areas of particles seen on the complementary face EF (Fig. 5-5 B). Two flagellar roots are seen and a part of the convex  $EF_2$  face of the chloroplastic envelope is also seen. fl, flagellar root. (x8300.) B, the particles (Fig. 5-5 A), highly magnified, on EF fracture face of plasma membrane. (x57,250.) C, the prints of the particles on PF fracture face of plasma membrane, highly magnified. (x57,250.) D, the convex  $EF_2$  fracture face of the outer chloroplastic membrane  $EF_2$  with areas of particles and windows showing PF<sub>1</sub> fracture face of the inner chloroplastic envelope. (x28,600.) Courtesy M. Lefort-Tran.

symbionts. In that case, the inner membrane envelope is equivalent to the plasma membrane of the cyanelle, and the other one is the perialgal vacuolar membrane homologue. In the euglenoid chloroplasts (Fig. 5-6), the outermost membrane corresponds to the vacuolar membrane of the host surrounding the double envelope of the symbiotic chloroplast. According to Lefort-Tran, the four-membraned chloroplast envelopes of cryptomonads may be compared with the actual *Paramecium-Chlorella* symbiosis; within an outer endomembrane (extension of the outer nuclear membrane) are present a eukaryotic endosymbiont with a plasma membrane and the double chloroplast envelope.

The inner membrane or lamellar system of algal chromatophores is a complex of appressed and interconnected flattened vesicles each designated a thylakoid. The arrangement of thylakoids differs in the various classes. Distinct grana are formed in the chromatophores of green algae and some prasinophytes but are not



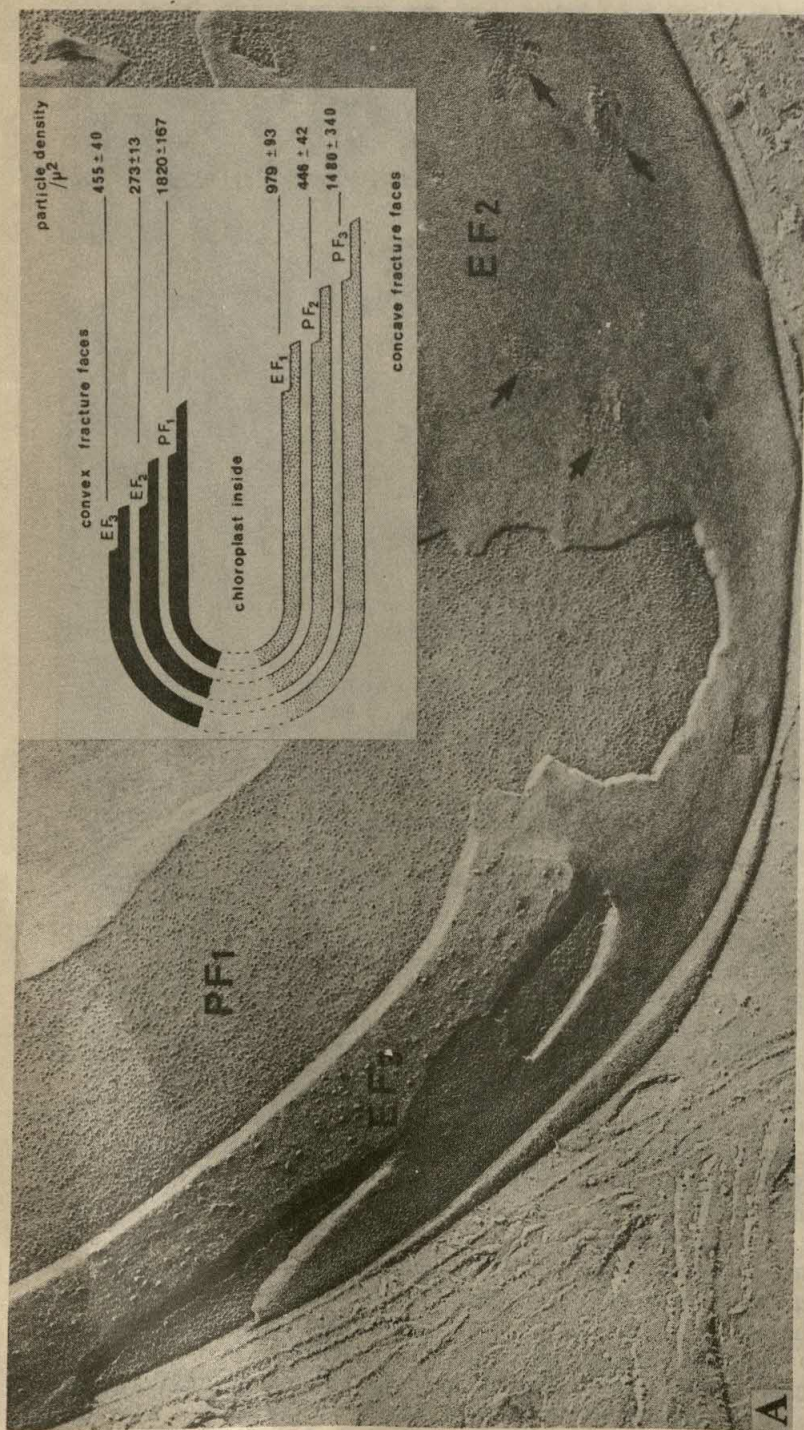


Fig. 5-6 Freeze fracture electron micrograph of *Euglena* chloroplast envelope. A, details of the three convex fracture faces. The inner membrane PF<sub>1</sub> is rich in particles and has the polarity of a plasma membrane. The two other faces (EF<sub>2</sub> and EF<sub>3</sub>) are exoplasmic and homologues of endomembranes. Prints of particles are visible on EF<sub>2</sub> (arrows). (x65,800.) *Insert:* Schematic sketch of fracture faces of three membranes of *Euglena* chloroplast envelope. B, view of concave fracture face PF<sub>2</sub> with organized areas of particles (arrows) complementary to those on EF<sub>2</sub> of Fig. 5-6 A. (x67,500.) Courtesy M. Lefort-Tran.



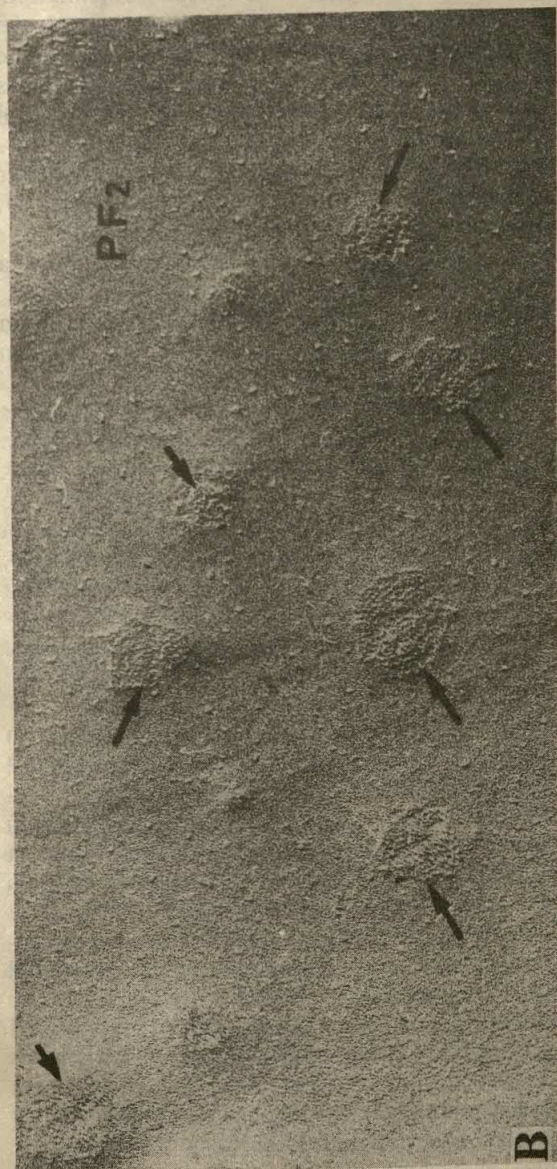


Fig. 5-6 Freeze fracture electron micrograph of *Euglena* chloroplast envelope. A, details of the three convex fracture faces. The inner membrane  $PF_1$  is rich in particles and has the polarity of a plasma membrane. The two other faces ( $EF_2$  and  $EF_3$ ) are exoplasmic and homologues of endomembranes. Prints of particles are visible on  $EF_2$  (arrows). (x65,800.) *Insert:* Schematic sketch of fracture faces of three membranes of *Euglena* chloroplast envelope. B, view of concave fracture face  $PF_2$  with organized areas of particles (arrows) complementary to those on  $EF_2$  of Fig. 5-6 A. (x67,500.) Courtesy M. Lefort-Tran.



formed in the chromatophores of algae belonging to other classes. In these remaining classes, however, groups of thylakoids may be appressed, sometime for the entire length of a chromatophore.

The composition and structure of photosynthetic membranes have been studied by electron microscopy of frozen fractured and etched chromatophore membranes during the last two decades. Branton (1966) reported that fracturing occurs in the hydrophobic phase within the membrane although the fracture face sometime passes between thylakoids. When splitting occurs within the membrane, particles of  $175 \times 90 \text{ \AA}$  are exposed. These particles have characteristic EF (face toward the lumen) and PF (face away from the lumen and toward the protoplasm) faces. These faces have partially embedded particles protruding from them. Later researches have shown that the EF particles constitute the site of Photosystem II, whereas the PF likewise constitute the site of PS-I activity (Bogorad, 1981).

The thylakoid membranes in *Scenedesmus* are about 11 nm thick and have a single row of spherical subunits having light cores and dark rims (Weier *et al.*, 1966). Neushul (1971) recorded two kinds of particles, 10 nm and 5 nm, in certain brown and red algal thylakoids. About 20-nm wide thylakoids are reported in *Batrachospermum* (Brown and Weier, 1970).

Chlorophyll is non-covalently bound to hydrophobic proteins. These proteins exist as multiprotein structural complexes embedded in the lipid phase of the chromatophore membranes of photosynthetic bacteria or the thylakoid membranes of eukaryotic chloroplasts. About 99% of the chlorophyll in the membrane functions simply as a light harvesting antenna. The remaining 1% is bound in unique reaction centre proteins in intimate contact with an electron acceptor. Chloroplasts contain two reaction centres acting in series. The reaction centre chlorophyll of PS-II absorbs near 680 nm and this system has a bound plastoquinone as the primary stable electron acceptor. In contrast, the reaction centre chlorophyll of PS-I absorbs at 700 nm and an iron-sulphur compound acts as the primary stable electron acceptor.

Staehelin and Arntzen (1983) have shown, on the basis of researches on such green algae as *Scenedesmus*, that a chlorophyll protein complex of chloroplast membranes undergoes reversible, lateral diffusion between appressed and non-appressed membrane regions (corresponding, respectively, to grana and stroma) under the control of a protein kinase. This diffusion is phosphorylation-dependent and elegantly regulates the amount of light energy that is delivered to the reaction centres of PS-I and PS-II, thereby regulating their rates of turnover. This kind of regulation explains why the two photosystems are physically separated in chloroplast membranes, the PS-II being located in granal regions and the PS-I in stromal regions. According to Staehelin and Arntzen, the feedback system involves the following steps. A membrane-bound kinase senses the rate of PS-II versus PS-I turnover via the oxidation-reduction state of the plastoquinone pool which shuttles electrons from PS-II to PS-I. If activated, this kinase adds phosphate (i.e., negative charge) to a granal pigment-protein complex. The change in its surface charge at a site critical for promoting membrane adhesion leads to increased electrostatic repulsion between the membranes, unstacking, and the lateral migration of the complex to adjacent stromal membranes.

Since long, cell biologists have been intrigued by the enigma of why do chloroplasts need grana? Or, why the chloroplast membranes of green algae and higher plants are spatially differentiated into grana and stroma? In fact, when sufficient light is available, grana are not required for the central function of



chloroplast membranes, viz., to trap radiant energy and produce stable high-energy chemical intermediates (ATP and NADPH<sub>2</sub>). But when the light conditions are limiting, then the stacked membranes (grana) are often correlated with increased efficiency of light trapping and utilization. When light limits photosynthesis, the primary adaptation of green plants is to increase proportionately the amount of chlorophyll a/b light harvesting complex in their thylakoids and concomitantly the proportion of stacked granal membranes (Staehelin and Arntzen, 1983).

Since the chlorophylls (a and b) associated with the light harvesting complex absorb at shorter wavelengths than the reaction centre chlorophylls of PS-II and PS-I, they can efficiently transfer excitation energy to both types of reaction centres by means of resonance energy transfer. Furthermore, several other types of excitation energy transfer also occur in chloroplast membranes (e.g., PS-II to PS-I transfer). We know that the PS-I reaction centre has the longest wavelength (700 nm) absorbing chlorophyll species and is also a better light trapper than PS-II. This raises the question as to why all the energy trapped by the different antenna systems does not end up eventually in PS-I. During evolution, this problem seems to have been solved by the spatial segregation of most of PS-II and chlorophyll light harvesting complex into grana, and most of PS-I into stromal membrane regions. This kind of spatial segregation of the two types of reaction centres constitutes an elegant check against excessive transfer of excitation energy from PS-II to PS-I. Since the environmental light conditions fluctuate widely, plants have developed a mobile antenna system that can, by lateral migration or diffusion, be variably partitioned between PS-II-rich grana and PS-I-rich stroma membranes. This constitutes an efficient mechanism to regulate energy distribution between the two photosystems (Staehelin and Arntzen, 1983).

Characteristic phycobilisomes occur outside the thylakoids (Figs. 5-7, 5-8) in red algae and cyanobacteria. They also are present in the Cryptophyceae but in this case their location is intrathylakoidal. Phycobilisomes are also described in Chapter 2 on cyanobacteria (see Figs. 2-2, 2-11).

Single thylakoids are characteristic of the Rhodophyceae and cyanobacteria. Thylakoids are arranged concentrically in *Porphyridium* and *Compsopogon*.

In the Cryptophyceae, the thylakoids are stacked in pairs (Fig. 5-9) and are never very closely appressed together. Paired thylakoids is also a general feature of the Chlorophyceae though some green algae have two to many thylakoids per lamella. In the Prasinophyceae, 2-4 thylakoids are present per lamella.

In the remaining classes, three-thylakoid lamellae are usual. Sometime, thylakoids from one lamella pass over to another lamella; such interconnections between lamellae are known to occur in certain Phaeophyceae, Bacillariophyceae, Chrysophyceae, Xanthophyceae, Chloromonadophyceae, Prasinophyceae, Chlorophyceae, and Haptophyceae (see Dodge, 1973).

A special kind of lamella called girdle lamella encircles the rim of the chromatophore and commonly lies parallel to the chromatophore envelope. Such girdle lamellae are known to occur in the Phaeophyceae, Chrysophyceae, Xanthophyceae, and Chloromonadophyceae. In *Vacuolaria*, the girdle bands are the outermost thylakoids just beneath the chromatophore envelope.

An extensive survey of chromatophore fine structure in the Dinophyceae (Dodge, 1975) has revealed the existence of five main types of chromatophores, viz., (1) those having parallel lamellae, e.g., *Ceratium* and *Gymnodinium fuscum*, (2) those with parallel lamellae plus also occasional peripheral lamellae, e.g., *Gonyaulax tamarensis*, (3) those having girdle lamellae and internal pyrenoid,



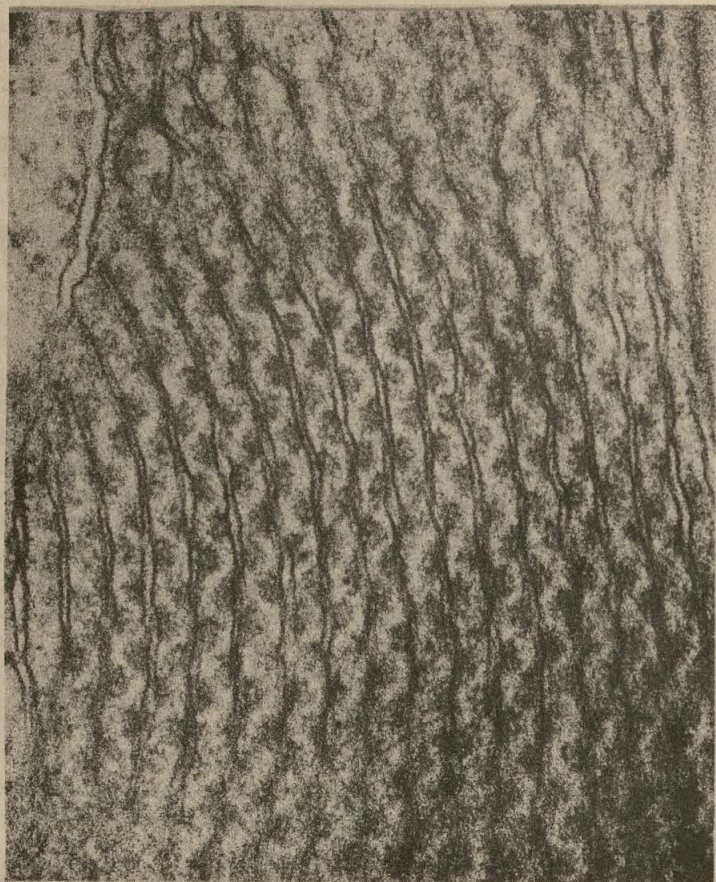


Fig. 5-7 Electron micrograph through section of chromatophore of *Porphyridium cruentum* (= *P. purpureum*), showing thylakoids and phycobilisomes. (x115,000.) Courtesy E. Gantt.

e.g., *Glenodinium foliaceum*, (4) those having fusion of thylakoids to the envelope and the internal pyrenoid, e.g., *Gymnodinium veneficum*, and (5) those having radially-arranged lamellae, e.g., *Gymnodinium simplex*.

The chromatophores of type (3) in *G. foliaceum* and in the binucleate alga *Peridinium balticum* greatly resemble those of the Chrysophyta and, instead of the usual carotenoid peridinin, they have fucoxanthin. Peridinin is also replaced by fucoxanthin in the chromatophores of type (4).

The chloroplasts in the Chlorophyceae and Prasinophyceae are the site of starch accumulation; in other classes, the reserve material forms outside the chromatophore in the cytoplasm.

*Euglenachloroplasts* have somewhat lower chlorophyll b content as compared to those of green algae and higher plants. The thylakoids are appressed in twos or threes over their entire length, forming long grana (Fig. 5-10). In contrast, those of dinoflagellates are close but not appressed.

In green algae and higher plants, the stable components of the PS-II seem to be the relevant reaction centres: they are the first basic building blocks of PS-II to be



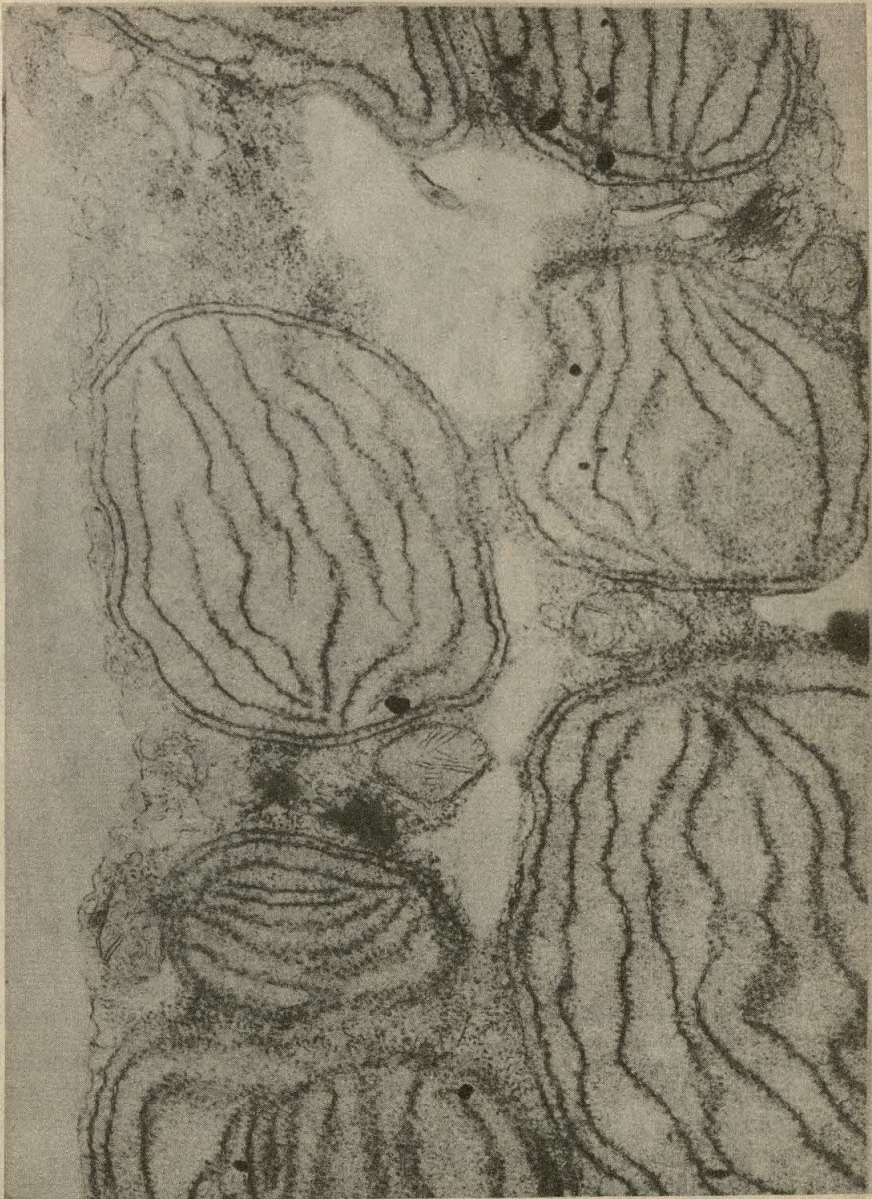


Fig. 5-8 Electron micrograph through part of cell of *Polysiphonia urceolata*, showing chromatophores with typically red-algal thylakoids. (x28,500.)  
Courtesy K. Ueda.

formed during plastid greening and are later completed by further development of the light-harvesting antennae. These plants adapt to different light intensities by regulating the size of their light-harvesting antennae. In contrast, the PS-II reaction centres of *Euglena* are more labile: they are the last components of the PS-II units to be formed during greening after completion of the development of the light-



harvesting antennae (Dubertret and Lefort-Tran, 1981). Dubertret and Lefort-Tran (1981, 1982) studied the plastid fine structure of *Euglena* that was greened under intermittent light (Fig. 5-3) and concluded that the PS-II units in this alga may occur as discrete individual entities in the photosynthetic membranes, and that their morphological expression corresponds to the exoplasmic fracture face particles.

Table VI summarizes our knowledge of the nitrogen metabolism and enzymology of chloroplasts and compares it with cyanobacteria.

Chromatophores not only contain the photosynthetic pigments but also DNA and RNA. The chromatophore genome consists of one or more linkage groups and contains genes for uniparentally transmitted traits in *Chlamydomonas*. Electron microscopic evidence for the presence of 25 Å thick DNA strands in the chloroplasts of *C. moewusii* was first given by Ris and Plaut (1962). Manning and Richards (1972) detected 40-μm long circular DNA molecules in chloroplasts of *Euglena gracilis*. Similar circular molecules also occur in *Chlamydomonas reinhardtii*. The chromatophore DNA molecule of *Euglena gracilis* is circular and double-stranded, having about 140 kbp. Its major RNA

transcripts are the 16S and 23S rRNAs. The chromatophore rRNA constitutes up to about 25% of the total cellular RNA in this alga. The chromatophore genome contains three tandemly repeated 5.6 kbp segments, each of which contains 16S and 23S rRNA genes (Gray and Hallick, 1978).

In *Vacuolaria*, the chromatophores contain ring-shaped DNA. Coleman and Heywood (1981) have studied the chromatophore DNA by fluorescence staining techniques. They have demonstrated that a single unbroken DNA ring is found per chromatophore. In this ring, the DNA is unevenly distributed and exists as a microscopically observable string of beads. Similar ring-shaped DNA molecules



Fig. 5-9 Electron micrograph of section through chromatophore of *Chroomonas* sp. An eyespot is also seen. (x39,000.) Courtesy E. Gantt.



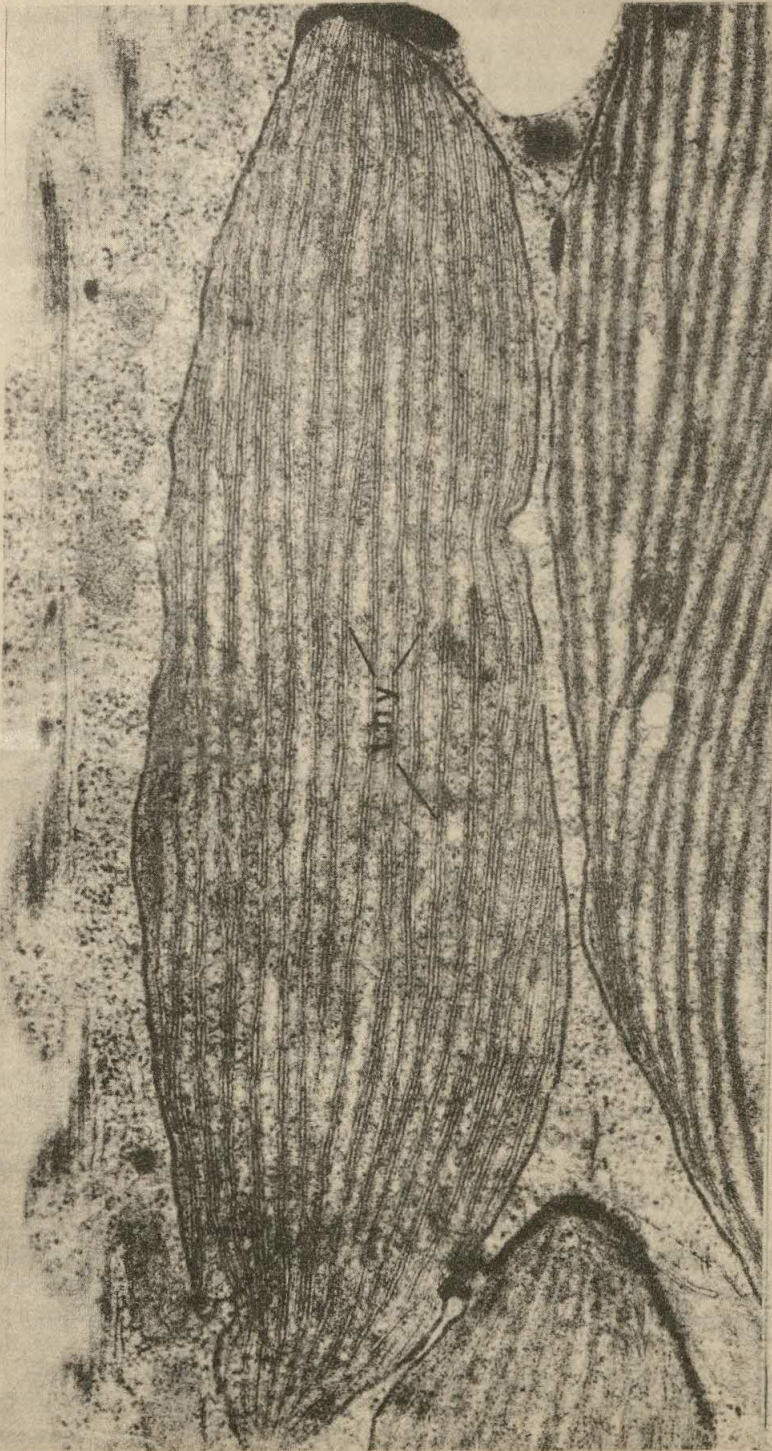


Fig. 5-10 Electron micrograph through chloroplast of *Euglena gracilis* fully differentiated under continuous light. Long thylakoids stretch along the full length of the chloroplast and appear to be appressed in twos or threes. thy, thylakoids. (x33,600.) Courtesy M. L'fort-Tran.



Table VI Comparison of nitrogen metabolism in cyanobacteria and chloroplasts (after Lea *et al.*, 1982)

Characteristic	Cyanobacteria	Chloroplasts
Nitrate reductase	Firmly bound to plasmalemma Ferredoxin dependent	If present, loosely bound to outer membrane NADH-dependent
Nitrite reductase	Membrane-bound, ferredoxin dependent	Located in stroma, ferredoxin dependent
Glutamine synthetase	Molecular weight up to 390,000; 8 subunits of 45,000 each No adenylation Reversibly deactivated	Molecular weight 600,000; 12 subunits of 50,000 each No adenylation Reversibly deactivated
Glutamate synthase	Ferredoxin dependent	Ferredoxin dependent
Amino acid dehydrogenases	Low levels of aspartic and alanine dehydrogen- ases	Low levels of glutamic dehydrogenase, some- time nil
Synthesis of alpha keto acids	Can synthesize all alpha keto acids but not via Krebs cycle	Cannot synthesize oxaloacetate or alpha keto- glutarate, but may synthesize pyruvate via ph- osphoenolpyruvate
Synthesis of carbon skeletons of amino acids	Can synthesize all amino acids Synthesis regulated by feedback inhibition, not repression	Can synthesize serine, aromatic and aspartate-derived amino acids Synthesis regulated by feedback inhibition, not repression



are present in chromatophores of diatoms, Chrysophyceae, Xanthophyceae, and Phaeophyceae (Taylor, 1976).

Many workers believe that chloroplasts may have evolved from ancient symbiotic photosynthetic prokaryotes (Gray and Doolittle, 1982). In some groups of algae, the chloroplast may have evolved from two successive symbioses, viz., (1) a prokaryote-eukaryote symbiosis, and (2) a eukaryote-eukaryote symbiosis (Ludwig and Gibbs, 1985). In the first symbiosis, a cyanobacterium entered a eukaryotic cell. In the second, even certain zooflagellates engulfed the products of the first symbiosis, and it is from this subsequent eukaryotic symbiont that the chloroplast of these flagellates may have evolved. Those algae which contain chloroplasts enclosed by four membranes appear to have obtained their chloroplasts in this way (Ludwig and Gibbs, 1985).

The chloroplast of *Chlamydomonas reinhardtii* is now believed to contain multiple copies of its genome (Forster *et al.*, 1980). This finding differs from the model of Sager (1977) who proposed that the chloroplast genes are located in two circular copies of the genome.

During gametic fusion, the chloroplasts of the fusing cells also fuse. During the subsequent maturation of the zygote, the paternal and maternal chloroplast-DNA molecules undergo different fates (Forster *et al.*, 1980), with maternal inheritance predominating. Any mutations in the chloroplasts are chiefly maternally-inherited. All meiotic products of most zygotes from a cross transmit only the alleles of chloroplast genes carried by the maternal parent.

Recently, Matagne and Hermesse (1980) have achieved somatic (protoplast) fusions between gametes of the same mating type. By this technique, they have studied the mode of chloroplast gene inheritance in the zygotes. Surprisingly, they found that about one-third of the somatic zygotes ( $mt^+/mt^+$ , or  $mt^-/mt^-$ ) transmitted chloroplast markers from both parents, rather than chiefly from the maternal ( $mt^+$ ) parent. The rest of the population was equally distributed between zygotes transmitting the plastid marker of one or the other parent exclusively. On the basis of these observations, Matagne and Hermesse have concluded that the general process of elimination of chloroplast alleles occurs independent of the presence of both maternal and paternal alleles in the cell. On the other hand, the preferential elimination of paternal chloroplast alleles does seem to depend upon heterozygosity at the *mt* locus (Matagne and Hermesse, 1980). Thus, whereas uniparental inheritance is chiefly unidirectional in sexually produced zygotes, it is bidirectional in the case of somatic zygotes.

In *Acetabularia mediterranea*, some chloroplasts, especially those near the cell apex, contain DNA, whereas others (mainly those at the basal part) lack DNA (Lüttke, 1981). Unlike the chloroplasts of *Chlamydomonas* and *Euglena*, those of *Acetabularia* (even those which have DNA) seem to lack any circular DNA molecules.

Lüttke has proposed that structural features of the chloroplast DNA and thylakoid arrangements may be responsible for the development of DNA-less chloroplasts during the course of chloroplast growth and development. In keeping with the presence or absence of DNA, it has been observed that the apical chloroplasts can actively multiply, whereas the basal ones divide very rarely, if at all.

Unlike nuclear DNA, the chloroplast DNA lacks 5-methylcytosine. Another characteristic feature of chloroplast DNA is the presence of two or more large repeated sequences. *C. reinhardtii* has two inverted repeats of 19 kbp each



(Rochaix, 1978). *Euglena gracilis* DNA contains three small tandemly repeated sequences of 5.6 kbp each (Gray and Hallick, 1978). In both these species, the repeats contain genes for chloroplast rRNAs. In *C. reinhardtii*, the gene for the large subunit of ribulose biphosphate carboxylase oxygenase enzyme has been located on the chloroplast genome (Malnoe *et al.*, 1979) (Fig. 5-11). Another noteworthy discovery is that in *Chlamydomonas* the gene for 23S rRNA contains an intron of 0.94 kbp (Rochaix and Malnoe, 1978).

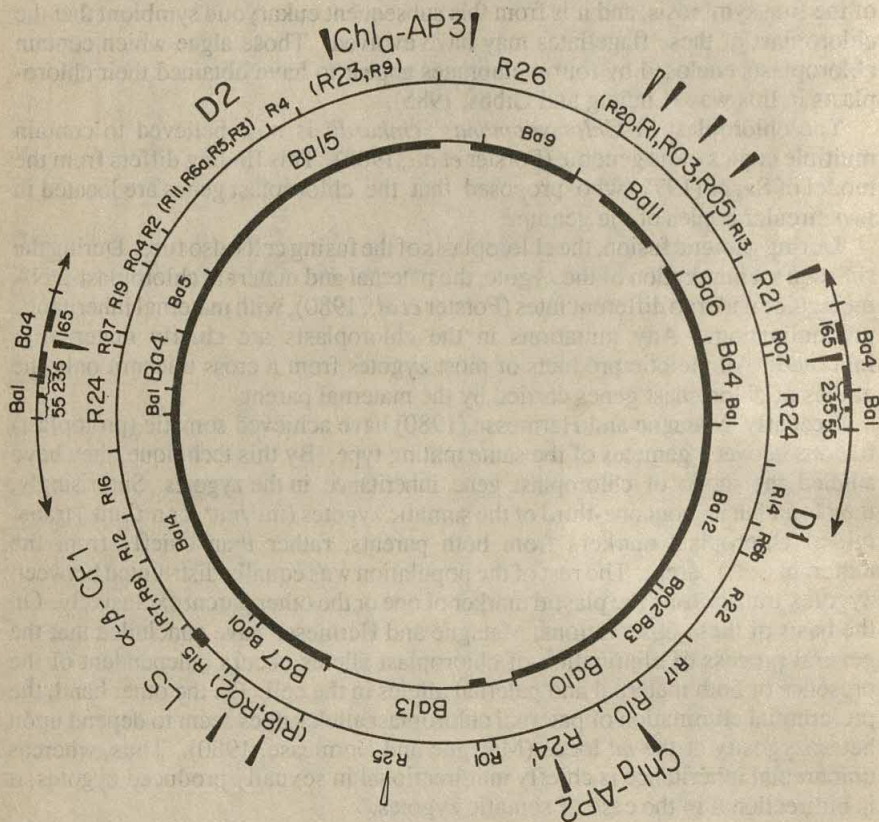


Fig. 5-11 *Chlamydomonas reinhardtii*. Chloroplast chromosomal map. R, *Eco*RI restriction endonuclease fragment (shown on outer circle); Ba, *Bam*HI restriction endonuclease fragment (shown on inner circle). Cloned regions of the genome are shown by thick line on the inner circle. The two rRNA genes are shown on the outside and the inverted repeats are indicated. Arrows indicate the position of 4S RNA genes and the fragments labelled with large letters hybridize to the 4S RNA. Fragment R15 contains the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase. (After Malnoe *et al.*, 1979.)

Researches during the last two decades have established that chromatophores divide, mature, differentiate for the production and storage of starch, oils or carotenoids; their genomes are small but not too small for studying the control of expression of identified genes (Bogorad, 1981).



Whereas in vegetative cells of *Acetabularia*, only a few of the chloroplasts contain one discernible DNA molecule per chloroplast, virtually all of the chloroplasts in developing cysts of this alga contain chloroplast-DNA, with as many as 9 nucleoids being present in each chloroplast (Coleman, 1979). The maximum number of nucleoids recorded per vegetative-cell chloroplast is two. This alga is ideally suited to the study of the cytoplasmic regulation of nuclear functions like nuclear DNA transcription and replication. It exhibits several diurnal rhythms, two of which often persist for long periods, viz., (1) the intracellular chloroplast migration rhythm and (2) the photosynthetic oxygen evolution rhythm.

Plastid genomes are histone-free, covalently closed circular DNAs. *Chlamydomonas* plastid DNA is about  $1.3 \times 10^8$  daltons with about 38 mol % of G + C content. *Euglena* plastid DNA has rather low (Table VII) G + C content but its size (40  $\mu$ m) resembles that of higher plant plastid DNAs. The plastid DNA of *Acetabularia* is very unlike the others; it has linear molecules up to 200  $\mu$ m long, with molecular weight around  $4 \times 10^8$ , which may be derived from large circular molecules, and Green (1977) has further reported small (4.3  $\mu$ m) covalently closed minicircular DNAs. The *Acetabularia* plastid DNA is equivalent to a medium sized bacterial genome.

Plastid DNAs contain few, if any, repetitive sequences. Gelvin and Howell (1979) found that up to 7% of the plastid DNA of *Chlamydomonas reinhardtii* consists of short inverted repeats distributed throughout the genome. Intervening sequences occur in the 23S rRNA gene of *C. reinhardtii* plastid DNA (Rochaix and Malnoe, 1978). This intervening sequence is remarkably similar to eukaryotic nuclear intervening sequences and exhibits some traits of transposons (Allet and Rochaix, 1979).

Some algal plastid genomes carry two or three copies of genes for rRNAs which are 23S, 16S, and 5S in size. In the plastids of *Euglena* and *Chlamydomonas*, rRNA genes are linked in the order 5'-16S-23S-5S-3', with spacers of 300 bp (*Euglena*) or 1700 bp (*Chlamydomonas*) separating the 16S and 23S genes (Gray and Hallick, 1978; Rochaix and Malnoe, 1978). In *Chlamydomonas*, there are some tRNA genes in this spacer (Malnoe and Rochaix, 1978). Unlike *Escherichia coli*, the *Chlamydomonas* 23S rRNA contains one intron and additional 3S and 7S RNAs. The inverted repeats define two regions of single-copy DNA, and loose clusters of tRNA genes are located in both of these regions (Wallace, 1982).

The plastid DNA of the chrysophyte *Olithodiscus luteus* contains three genomic elements 44  $\mu$ m, 22  $\mu$ m, and 11  $\mu$ m long, giving a total genome complexity of 154 megadaltons (Aldrich and Cattolico, 1979). This genome (as also the genome of *Vacuolaria*) is packaged in ring-shaped beaded structures. In *Gonyostomum*, the plastid DNA is 55  $\mu$ m in circumference (Coleman and Heywood, 1981).

In recent years, certain fluorochrome dyes which fluoresce upon binding with DNA have been used to great advantage in algae. By using this technique, Coleman (1978) has studied the chloroplast DNA in Volvocalean algae. She has found that the plastid DNA organization in these flagellates ranges from isolated condensed areas, called nucleoids, to beaded strings, to diffuse strands. Using an improved method of DAPI (4',6-diamidino-2-phenylindole) staining, Kuroiwa and Suzuki (1980) studied the chloroplast nucleoids in several algae and recognized the following five types based on differences in the distribution, size, or number of the nucleoids:

(1) SN type Chloroplasts have small, uniformly dispersed nucleoids, e.g., *Cryptomonas*, *Euglena gracilis*, and many green algae.



Table VII Comparison of some cyanobacterial and plastid DNAs\* (after Trench, 1982; Bogorad, 1982)

System	Genome size (daltons x 10 <sup>-9</sup> )	Buoyant density (g/cm <sup>3</sup> )	G + C (mol %)	Number of genome copies	Repeated sequences (kbp)	
					Inverted	Tandem
PLASTIDS						
<i>Chlamydomonas reinhardtii</i>	0.194	1.695	35.7	82	19 x 2	
<i>Chlorella pyrenoidosa</i>	0.210	1.687	27.6	19		
<i>Euglena gracilis</i>	0.094-0.180	1.685	25.5	67-80		5.6 x 3**
CYANOBACTERIA						
<i>Anacystis nidulans</i>	2.27	1.715	56			
<i>Gloeocapsa alpicola</i>		1.694	35			
<i>Cyanocyta korschikoffiana</i>	0.117	1.695-1.716	35.7-57	60		

\*All these DNAs form circular chromosomes.

\*\* These repeats are located close together.



(2) CN type One or a few nucleoids are present in the central part of the chloroplast, e.g., *Acetabularia calyculus* and *Glaucocystis nostochinearum*.

(3) CL type A large ring-shaped nucleoid is present inside the girdle lamella, e.g., many diatoms and brown algae and *Cyanidium caldarium*.

(4) SP type Numerous small nucleoids form a shell around a pyrenoid within the chloroplast, e.g., *Cladophora glomerata* and *Bryopsis plumosa*.

(5) PS type Nucleoids lie scattered along the periphery of the chloroplast, e.g., *Gelidium amansii* and *Symphyocladia latiuscula*.

Coleman (1985) has classified algae into two broad categories on the basis of their plastid DNA. Type 1 algae have nucleoids of variable size and morphology, lying scattered throughout the plastid. Distribution: Rhodophyta, Dinophyta, Chlorophyta, Cryptophyta, Prymnesiophyceae, and Eustigmatophyceae.

Type 2 algae have a ring nucleoid, beaded or unbeaded, lying just within the girdle lamella. Distribution: Phaeophyta, Bacillariophyta, Raphidophyceae, some Tribophyceae, and several Chrysophyceae.

Hashimoto (1985) has postulated that plastid nucleoids are associated with thylakoid membranes at specific sites. This can explain the oft-observed close correlation between the changes in spatial arrangement of plastid nucleoids and the development of the inner membrane systems of plastids. On the basis of these observations, Hashimoto suggested that the different spatial arrangements of plastid nucleoids are determined by their attachment to some specific sites of the thylakoid membranes lying at different regions within the developing plastid.

Besides microscopic examination of DNA by fluorochrome staining, molecular genetical techniques have been used to study plastid DNA in some algae. The main approach to direct analysis of such DNA involves the use of restriction endonucleases which recognize specific nucleotide sequences on the DNA molecule and cleave the genome into distinctive segments. Variations in fragmentation patterns between different species, or differences between wild-type and mutant DNAs reflect differences in nucleotide sequences in their genomes. These differences may or may not lead to any observable phenotypic variations. The plastid DNA has been found to be quite amenable to such "restriction analysis". When this DNA is subjected to digestion with restriction endonucleases, some distinctive fragments are produced which can be separated on the basis of their size. It is possible to isolate such individual DNA segments and to use them in DNA/DNA or RNA/DNA hybridizations, or can be attached to plasmids of *E. coli* and cloned for future use. Helling *et al.* (1979) have used this kind of approach to analyze the chloroplast RNA genes of *Euglena gracilis* var. *bacillaris*. The order of eight of the 29 *EcoRI*-generated fragments of chloroplast DNA was determined. Three sets of rRNA genes aligned sequentially in the same orientation were found to constitute a part of this region. *E. gracilis* chloroplast contains circular DNA molecule of 138 kbp length. Out of this, 17 kbp is needed to code for the three sets of rRNA genes (Gray and Hallick, 1978). Another 2 kbp codes for some 26 tRNA genes. The remaining DNA codes for ribulose biphosphate carboxylase and certain other proteins.

When compared to *Chlamydomonas reinhardtii*, the chloroplast genome of *Euglena* differs strikingly. The plastid DNA of *Chlamydomonas* has two copies of rDNA in inverted repeats and one separated by a long segment of the plastid DNA. In another similar study of the same system, Chelm *et al.* (1979) studied the gene regulation during chloroplast development. RNAs were isolated from



*Euglena* at different developmental stages and then characterized by their hybridization to specific restriction fragments. This approach enables the investigator to differentiate between those genes that are expressed at all times and others which are specific to some particular stage of development. Chelm *et al.* succeeded in delineating a temporal sequence of RNA transcription from defined regions of the chloroplast genome. They have reported the presence of the following types of RNA among the different genes or transcription units: (1) those which can be detected throughout development, (2) those that are induced at the start of development, (3) those which remain repressed early in development, and (4) those which are induced rather late in development.

The first known algal system in which one can readily obtain the progeny of an interspecific cross which is recombinant for both chloroplast and nuclear genes of the two species is the recently described cross between *Chlamydomonas eugametos* and *C. moewusii* (Lemieux *et al.*, 1981). These workers have used species-specific patterns of restriction fragmentation to directly follow the inheritance of plastid DNA in this interspecific cross. The cross yielded some rare, viable progeny cells which contained: (1) some restriction fragments common to the plastid DNA of one species, (2) some that were common to that of the other species, and (3) a novel, new category of fragments not found in either of the two mated species. This third category points to the first direct molecular evidence for genetic recombination between plastid DNAs of two species. This kind of system raises the hope that the complex communication occurring between the nucleus and the plastid may soon be understood in molecular terms.

Chloroplasts as well as mitochondria contain genetic information and synthesize some proteins (Table VIII) but they also depend to a considerable extent on the import of polypeptides synthesized in the cytoplasm. During the past few years, many workers have studied the selective uptake of different classes of proteins into chloroplasts and mitochondria, and the general conclusion that has emerged is that protein import into these organelles is a post-translational process not coupled to protein synthesis (Kreil, 1981). The first precursor of an organelle protein was discovered in studies on the biosynthesis of the small subunit of the major chloroplast protein ribulose biphosphate carboxylase. Dobberstein *et al.* (1977) showed in *Chlamydomonas reinhardtii* that a precursor for this protein is synthesized on free polysomes and is then processed after chain completion by an endoprotease present in the alga.

**Table VIII** Sites of synthesis of some plastid proteins (after Kirk and Tilney-Basset, 1967)

Plastid protein	Algae	Presumed site of synthesis
Ferredoxin	<i>Chlamydomonas</i> <i>Euglena gracilis</i>	Cytoplasm
Ferredoxin-NADP reductase	<i>Chlamydomonas</i>	Cytoplasm
Cytochrome f553	<i>E. gracilis</i>	Cytoplasm
Fatty acid synthetase	<i>E. gracilis</i>	Plastid

Algal plastids show a close affinity to oxygenic photosynthetic prokaryotes (cyanobacteria and *Prochloron*) and seem to have had a polyphyletic origin. The



strong similarities in the physiological, biochemical, and other properties of plastids and cyanobacteria suggest that the cyanobacteria were ancestral to the plastids (Gray and Doolittle, 1982). Recent researches reveal that the plastids of the Rhodophyta and Cryptophyta may safely be regarded as having a cyanobacterial origin. On the other hand, the plastids of the Chlorophyta and higher plants may have originated from *Prochloron*-like ancestors. The plastid DNAs of eukaryotic algae have been found to be more variable in size and organization than those of vascular plants (Wallace, 1982).

Paques and Brouers (1981) have studied the phototactic behaviour of chloroplasts of *Acetabularia mediterranea*. Chloroplast phototaxis in this species is a photoenzymatic process and neither chlorophylls nor phytochromes are the receptors involved in the phototactic response but some flavines may act as the receptors. The chloroplasts migrate rhythmically along the stalk, the migration being directed toward the cell's apex in the morning and toward the rhizoids in the evening. At dawn or dusk, the chloroplasts migrate in transversely disposed bands. This kind of banding of plastids is particularly characteristic of the younger plants of *Acetabularia*.

Plastids often show intracellular orientation in response to light. In *Vaucheria*, an actin-myosin interaction seems to be the mechanical basis for plastid photo-orientation (Haupt, 1982). In *Mougeotia*, the plastid movement is mediated by phytochrome which is present in the chloroplasts. Electron microscopy of *Mougeotia* has revealed bundles of filaments running in between the chloroplast envelope and the plasmalemma. The diameter of these filaments is 5–10 nm. This suggests that they may be actin microfilaments (Wagner and Klein, 1981). Regulation of actin activity seems to be tightly coupled to Pfr (Haupt, 1982). It is thought that calcium redistribution connects Pfr to actin activity.

Chloroplast arrangements are not necessarily always related to light direction. In *Caulerpa* and *Acetabularia*, chloroplasts move together with the cytoplasm and, during the day, are more concentrated in the upper parts of the thallus, whereas they move to the basal part at night. These movements are not directly determined by light but rather are controlled by circadian rhythms.

The light-induced orientation of chloroplast in *Mougeotia* is controlled by a tetrapolar gradient in the phototransformational state of the photochromic pigment phytochrome. Wagner (1981) has proposed that a sliding interaction of actin with myosin, coordinated by a phytochrome-dependent regulation of plasmalemma-bound anchorage proteins to actin, may be involved in the precise reorientation of the chloroplast.

Unlike the plastids of most higher plants, those of most algae divide by fission (in most higher plants, mature plastids do not divide but the proplastids do). The algal plastids formed by fission grow to their mature size without much morphogenetic change. Some red, green, and other algae, however, do contain proplastids as well. It is interesting that despite the marked structural differences among the mature plastids in the different algal classes, the proplastids of most algae are quite similar. These proplastids are spherical or amoeboid, surrounded by envelopes, and have their inner membranes commonly arranged as single peripheral girdles (Klein, 1982).

The occurrence of certain prolamellar bodies in the young plastids of higher plants is well known (Kirk and Tilney-Basset, 1967). Fairly similar tubular complexes are known to occur in the proplastids and plastids of *Anabaena* (cells), *Chlorella*, *Chlamydomonas* and *Caulerpa* spp., *Euglena gracilis*, and *Batra-*



*chospermum moniliformis*. These prolamellar bodies or tubular complexes generally tend to form as a transient response to darkness but, in *Caulerpa* and *Halimeda*, they are rather permanent (Calvert *et al.*, 1976), being formed irrespective of the light conditions.

Various phycologists have placed the anomalous and enigmatic alga *Cyanidium caldarium* in the Chlorophyta, Cyanophyta, or Rhodophyta. Kremer and Feige (1979) have suggested that the "chloroplast" of *Cyanidium* is probably a cyanelle. This organism is eukaryotic, having phycocyanin. It grows in thermal springs. In a recent electron microscopic study, using the improved glutaraldehyde fixation method, Ueda and Yokochi (1981) have found that its chloroplasts have 8–10 unstacked thylakoids within their double-membraned envelope. The ultrastructural features of mitochondria, endoplasmic reticulum, microbodies, vacuoles, nuclear membranes, and ribosomes were found to be quite patently eukaryotic. The chloroplast fine structure of *C. caldarium* strongly resembles that of a red algal chromatophore; in both cases, thylakoids occur unstacked within the double-membraned envelope and a girdle thylakoid is also found in *Cyanidium* as well as in red algal chromatophores (Young, 1979).

The thylakoids of *Prochloron* (Prochlorophyceae) resemble those of the green algae in being differentiated into stacked and unstacked regions (Giddings *et al.*, 1980). There is a considerable ultrastructural resemblance between the chloroplasts of *Prochloron*, green algae, and the higher plants. Whereas cyanobacterial thylakoids have phycobilisomes, the thylakoids of *Prochloron* lack them. The light-harvesting complex of *Prochloron* also differs considerably from that of cyanobacteria but shows a strong resemblance with that of green plants. Another difference between *Prochloron* and cyanobacteria is the thylakoid stacking in the former and its absence in the latter. In red algae also, the thylakoids are unstacked.

In some algae belonging to the Tribophyceae, Chrysophyceae, Phaeophyceae, Bacillariophyceae, and Chloromonadophyceae, the chloroplast envelope is surrounded by a sheath of endoplasmic reticulum. This sheath is continuous with the nuclear envelope (Fig. 5-12).

## PYRENOIDS

These are integral parts of chromatophores in many algae (Fig. 5-13). They are completely absent in the Cyanophyceae. Virtually all green algae possess pyrenoids. In other classes, some members may lack pyrenoids. The ground matrix of pyrenoids is proteinaceous. In green algae, starch is deposited around this matrix. In other classes, other food reserve or storage product may be laid down around the matrix.

Pyrenoids are broadly classified into the following categories.

**Simple and internal** It is a simple fusiform or flattened body present across the centre or along one side of the chloroplast. No thylakoids enter the pyrenoids in several members of the Dinophyceae and a few species of the Haptophyceae and Chrysophyceae. Most Haptophyceae and diatoms have a pyrenoid in which thylakoids do enter and run through the matrix (Table IX).

**Compound and internal** These are large and only one or two are generally found per cell. In *Porphyridium cruentum* and *Vaucheria sphaerospora*, the pyrenoid has randomly arranged thylakoids. Certain dinoflagellates, e.g., *Prorocentrum*, many Xanthophyceae, and isolated members of a few other algal classes have



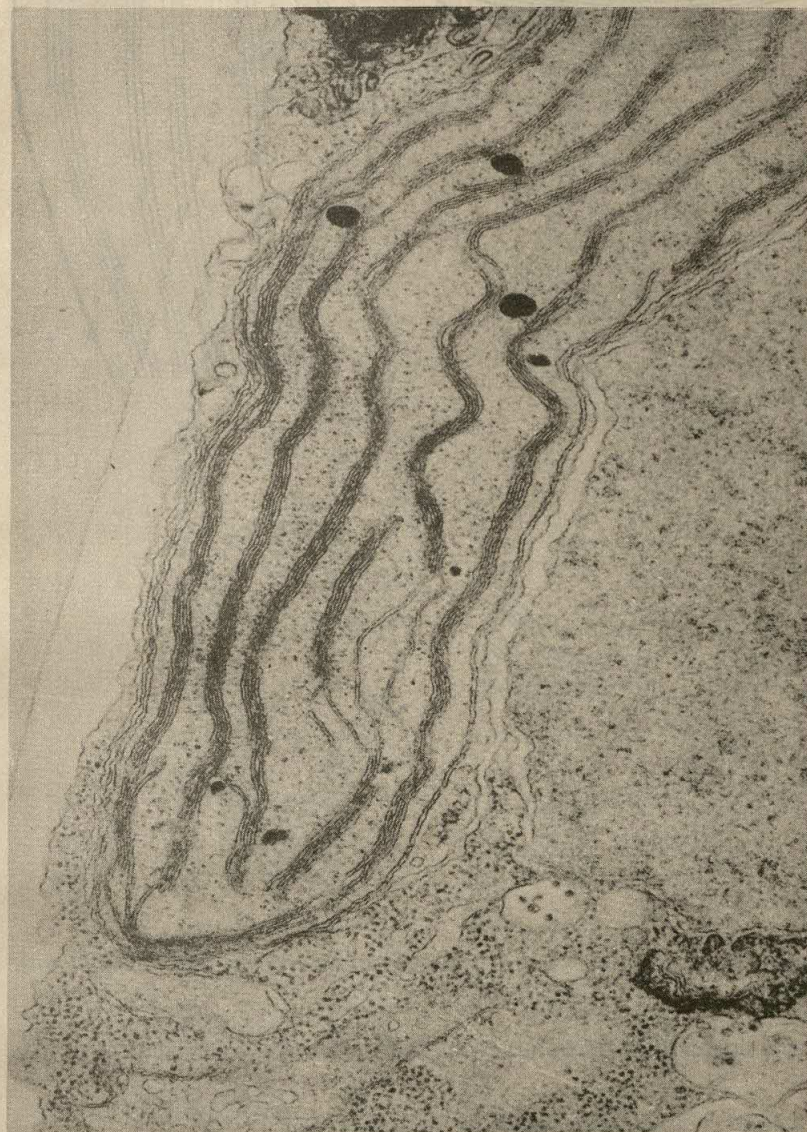


Fig. 5-12 Electron micrograph of section through part of cell of *Scytosiphon lomentarius*, showing the extension of the nuclear envelope around the whole chloroplast. (x42,300.) Courtesy K. Ueda.



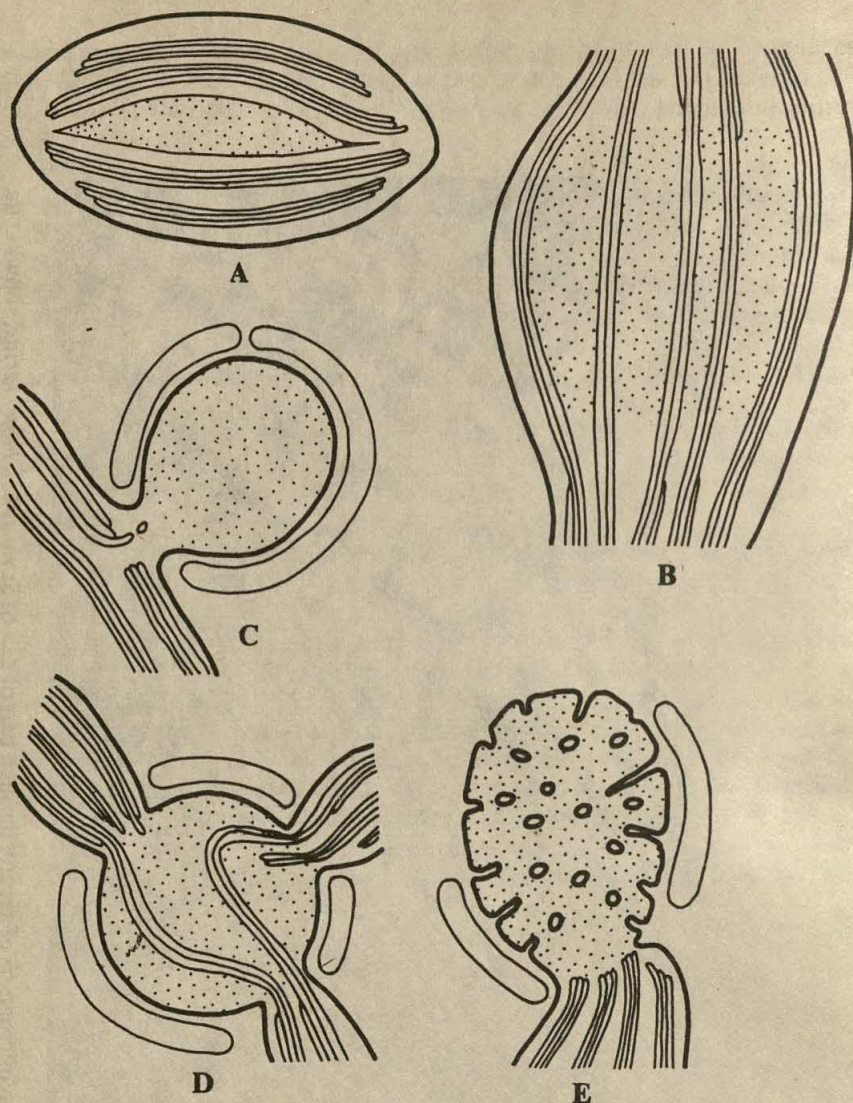


Fig. 5-13 Diagrammatic sketches to illustrate five types of pyrenoid met with in algae.

compound internal pyrenoids in which the lamellae are arranged in a parallel fashion.

**Stalked** This kind protrudes out from the plastid while still remaining attached to it. Single-stalked pyrenoids occur in many representatives of the Cryptophyceae, Dinophyceae, and Phaeophyceae (in all these cases, no thylakoids enter the matrix), whereas in the case of *Trachelomonas*, *Chrysochromulina*, and *Colacium*, lamellae do enter the pyrenoid. Multiple-stalked pyrenoids occur in some species of euglenoids, dinoflagellates, cryptophytes, and red algae.



Table IX Comparison of pyrenoid and eyespot types and flagellation in eukaryotic algae

Class	Pyrenoids	Eyespots	Flagella
Chlorophyceae	Occur in starchy plastid; invaginated single central or compound type	Part of chloroplast but not associated with flagella	2 or more, smooth
Tribophyceae	Internal compound type or stalked	Part of chloroplast but not associated with flagella	1 smooth, 1 with stiff hairs
Bacillariophyceae	Internal simple or internal compound	Male gametes have eyespots of Tribo- phycean type	1, with stiff hairs
Phaeophyceae	Stalked	Absent	1 smooth, 1 with stiff hairs
Rhodophyceae	Internal simple, internal compound, or stalked	Absent	Absent
Euglenophyceae	Internal compound, or stalked	Independent of chloroplast but adjacent to flagella	1 or more, with fine hairs; a hollow paraflagellar rod may occur
Chrysophyceae	Internal simple or internal compound type, or invaginated in non-starchy chloroplast	As in Tribophyceae	1 smooth, 1 with stiff hairs
Dinophyceae	Internal simple or internal compound type, or stalked; or invaginated in non-starchy plastid	Diverse, complex, well-developed eyespots are present	1 smooth, 1 with fine hairs; a solid paraflagellar rod may occur
Cryptophyceae	Stalked; invaginated in non-starchy plastid	Broadly similar to those in Chlorophyceae	2, with stiff hairs
Haptophyceae	Internal simple or internal compound, or stalked		2, smooth; haptonema present
Eustigmatophyceae	Stalked	Broadly similar to those in Euglenophyceae	1, with stiff hairs
Prasinophyceae	Invaginated in starchy chloroplast	As in Chlorophyceae	2 or more, with scales and short hairs



**Invaginated** This kind is penetrated by invaginations from the nucleus or the cytoplasm. Examples are *Rhodella maculata*, *Cryptomonas* spp., *Chrysamoeba radians*, *Oedogonium*, *Bulbochaete*, and *Platymonas*.

**Embedded** This kind is entirely embedded in a starch-containing chloroplast. Several green algae have this type of pyrenoid surrounded by a starch sheath, but the pyrenoid of some species, e.g., *Carteria acidicola*, lacks the starch sheath.

Recent research on isolated pyrenoids of *Eremosphaera* has suggested that the pyrenoid may serve as a storehouse of certain enzymes and that these may be utilized by newly-formed cells.

In most algae, pyrenoids seem to undergo division with the daughter pyrenoids being passed on to daughter cells. The stalked pyrenoids in some brown algae do not divide but bud off new pyrenoids from the base of the parent pyrenoid just before cell division. A third phenomenon occurs in many green algae in which the pyrenoids tend to disappear at cell division and later reappear in the daughter cells.

In some cryptophytes, e.g., *Chroomonas salina*, the nucleomorph in interphase cells may be embedded in an invagination of the pyrenoid.

In the Eustigmatophyceae, pyrenoids are rare but, when present, occur both in vegetative cells and motile stages. Three-thylakoid lamellae enter the pyrenoid matrix.

The Bangiophyceae tend to have single stellate or lobed chromatophores containing a single pyrenoid but lacking an inner limiting disc. Most Florideophyceae possess several discoid chromatophores per cell. These chromatophores have an inner limiting disc but lack pyrenoids. An exception is *Nemalion* where both pyrenoids and inner limiting disc coexist together.

In the Prasinophyceae, the pyrenoid is penetrated by extensions from the cytoplasm or even the nuclear envelope.

## GOLGI APPARATUS

The Golgi complex includes a morphologically heterogeneous series of membrane-limited components which are interposed between the ER and the plasmalemma. Its constant and characteristic structural component is a stack of smooth-surfaced cisternae (Fig. 5-14), which commonly have flattened, plate-like centres and more dilated rims. Sometime the cisternae may be curved, with one side of the stack being oriented toward the rough ER and the other facing the plasma membrane. The former side is generally associated with small vesicles, whereas the latter side is similarly associated with secretory cells or vacuoles. In addition to its own structural elements, the Golgi complex may be surrounded and crowded by coated vesicles, lysosomes, centrioles, microtubules, or other cell structures.

The central function of the Golgi complex is in secretion. Concentration and packaging of secretory products in most cells occur in the dilated rims of some cisternae. Concentration results in the formation of a storage granule with a condensed content and a membrane acquired in the Golgi complex. Passage of secretory products through the Golgi complex is obligatory and involves extensive modification and transfer to a membrane container which fuses with the plasmalemma at the time of exocytosis (Farquhar and Palade, 1981). Another major function relates to the post-translational modification of glycoproteins. According to Farquhar and Palade, the Golgi complex sorts out secretory, lyso-





Fig. 5-14 Part of cell of *Micrasterias crux-melitensis*, showing dictyosomes. (x37,500.) Courtesy K. Ueda.

somal, and certain membrane proteins, and directs them to their correct destinations.

Golgi bodies are present in all algae except the cyanobacteria and *Prochloron*. Their number per cell may vary from one (e.g., *Micromonas*) to many (e.g., *Pinnularia*). In *Gonyaulax* sp., the Golgi bodies are disposed in a ring around a central vesicle in close association with the nucleus. In these algae, the major area of the cell is occupied by fibrous vacuoles with or without a dense granular centre (Gaudsmith and Dawes, 1972). Coated vesicles are occasionally seen in the cell centre within the ring of Golgi bodies.

In *Scenedesmus acuminatus*, dictyosomes are located close to the nuclei, with dictyosomal cisternae being aligned parallel to the nuclear membrane (Ueda and Noguchi, 1986). The cisternae are of three kinds, viz., (1) proximal, having wide lumina, (2) distal, thin cisternae, and (3) intermediate cisternae. These three types probably correspond morphologically to the successive developmental stages of the cisternae, from immature to mature.

The division of dictyosomes in the desmid *Micrasterias* has been observed by Noguchi (1983). In *M. crux-melitensis* and *M. pinnatifida*, the dictyosomes divide synchronously with the premitotic stage, thereafter separating into two groups, each going to a daughter cell. One day before cell division, a cell of *M. crux-melitensis* contains 224–226 dictyosomes. Mitosing cells have about double this number.



Vegetative cells of the Eustigmatophyceae contain Golgi bodies but the reproductive (motile) cells lack them.

The Golgi apparatus participates in the biogenesis of certain iridescent bodies found in the vegetative cells of brown algae such as *Cystoseira* (Pelligrini and Pelligrini, 1982). These iridescent bodies are vesicles containing dense, proteinaceous globules which include some polysaccharides, and may be equivalent to specialized vacuoles. Iridescent vacuoles seem to derive from the Golgi apparatus either by formation of vesicles that are pinched off from cisternal margins, or directly by inflation of the most distal cisternae.

Golgi bodies are the most distinctive and most highly organized of the subcellular organelles of red algal cells. The Golgi-derived vesicles play a major role in cell wall formation and in the production of various internal storage products. Three chief kinds of vesicles found in red algal Golgi bodies are fibrillar, cored, or striated. The Golgi bodies of *Corallina* and *Jania* are unique in that there is a close apposition of the membranes of adjacent cisternae. In large and highly active Golgi bodies, the membranes of cisternae are in close apposition at the centre of the stack. This is a feature unique to the red algae. There are also some indications of the occurrence of fusions between the outer portion of the adjacent cisternal membranes.

Wetherbee and West (1976) have reported the existence of a unique kind of Golgi apparatus in *Polysiphonia novae angliae*. As in other red algae, the Golgi apparatus in this species initially produces certain vesicles which are involved in wall formation. During the early stages of carpospore development, crystalline structures, called striated vesicles, arise within rough endoplasmic reticulum. These striated vesicles later decrease in size and ultimately disappear. However, the disappearance of striated vesicles is followed by the emergence of certain Golgi-derived, crystalline vesicles in the cytoplasm and this is a unique feature observed in this species. The ultrastructures of striated vesicles and Golgi-derived vesicles are quite different. No such dimorphic vesicles seem to be present in the Golgi apparatus of any other organism. In *P. novae angliae*, the Golgi apparatus seems to have a role in the transformation and turnover of protein within developing carpospores.

In the prasinophytes, the dictyosomes are located peripherally to the basal bodies, anterior to the nucleus. The dictyosomal cisternae are the site of formation of flagellar and cell body scales in these algae.

In most diatoms, the Golgi bodies are localized exclusively in the plasma packet containing the nucleus, but in *Synedra* they lie in a plasmatic ribbon-like structure extending over the whole length of the cell.

The Golgi apparatus plays an important role in the formation and concentration of cell wall materials. These materials are transported within Golgi-derived vesicles to the developing cell wall. Certain pectic substances and hemicelluloses are synthesized in the dictyosomes and transported in Golgi vesicles to the cell wall. In algae, Golgi apparatus is known to play a role in the formation of the framework as well as the matrix material of cell walls (Manton, 1967; Hawkins, 1974). The cell walls of most red algae consist of a fibrillar component and a mucilaginous component. The latter usually forms a non- or para-crystalline matrix in which the fibrillar components lie embedded (Hanic and Craigie, 1969).

Tsekos (1981) has studied the fine structure of the Golgi apparatus during carposporangial development in *Gigartina teedii*. He found pronounced changes in size and structure in the dictyosomes of developing carposporangia. Normal



dictyosomes (stacks of 5–7 straight cisternae) occur in gonimoblasts and auxiliary cells, whereas young carposporangia have hypertrophied Golgi cisternae with fibrillar contents. Cisternae give rise to secretory vesicles, and mucilage sacs are derived from dilating endoplasmic reticulum or membranaceous formations of lysosomal nature. The mucilage sacs disappear in mature carposporangia. During the wall development of carposporangia, multivesicular lomasome-like bodies are released through the plasmalemma and may contribute to the plasticity of the expanding cell wall.

Very active dictyosomes with a semi-circular profile occur in older carposporangia; these produce cored vesicles (having electron opaque centres possibly containing glycoproteins).

The dictyosomes of older carposporangia are made up of a polarized stack of 7–11 cisternae, some of which contain conspicuous granules. According to Tsekos (1981), materials polymerize within the Golgi apparatus and the inflated Golgi-derived vesicles participate directly in the formation of the fibrillar network of the carpospore wall in this alga.

The Golgi apparatus plays a dual role in freshwater Chloromonadophyceae. Firstly, it functions in osmoregulation. Secondly, it produces some material that is discharged by the trichocysts (Heywood, 1980).

The Golgi bodies and lysosomes are examples of organelles that do not contain any DNA.

## MITOCHONDRIA

These powerhouses of the cell occur in all eukaryotic algae. They are involved in electron transport, oxidative phosphorylation, and release of energy.

*Pyrodinium bahamense* has a unique type of circular or semi-circular mitochondria (Gaudsmith and Dawes, 1972).

*Chlamydomonas* cells can have several mitochondria per cell. In *C. reinhardtii*, the volumes of mitochondria also vary from  $0.03 \mu\text{m}^3$  for tiny mitochondria to small ( $0.04$ – $0.09 \mu\text{m}^3$ ), medium ( $0.1$ – $0.5 \mu\text{m}^3$ ), large (over  $0.5 \mu\text{m}^3$ ), and giant (about  $1 \mu\text{m}^3$ ) mitochondria. Up to 25 mitochondria can occur in a single cell (Blank and Arnold, 1980). Some of these mitochondria may be branched, whereas others can fuse together to form a network that lies in the cell centre.

In the Prasinophyceae, the mitochondria are few in number and have flattened cisternae; they occur adjacent to the nucleus on the interior side of the chloroplast.

In the Cryptophyceae, mitochondria occur in the cytoplasm mostly near the flagellar bases. Generally, an unbranched, vermiform mitochondrion is found in *Hemiselmis rufescens*, whereas *Chroomonas* and species of *Cryptomonas* have variously branched and prominent mitochondria. The mitochondria of *Chroomonas* have a complex, interconnected peripheral and central reticulum. The mitochondrial cristae of most cryptophytes are characteristically flattened and finger-like (Santore and Greenwood, 1977). When *Euglena gracilis* Z is grown in the dark, it forms giant mitochondria (Fig. 5-15) (Lefort, 1964). According to Lefort (1964), mitochondria may be dynamic rather than static organelles and are capable of continued fragmentation and fusion. In certain green algae also, numerous small mitochondria distributed throughout the cytoplasm are seen during the cell division phase, but they form a subpellicular network during the non-dividing phase (Calvayrac *et al.*, 1972). Ledoigt, Calvayrac, and Lefort-Tran (personal communication) made the important observation on synchronized cells of *Euglena* that the mitochondrial DNA replicates at the same time as the nuclear DNA.



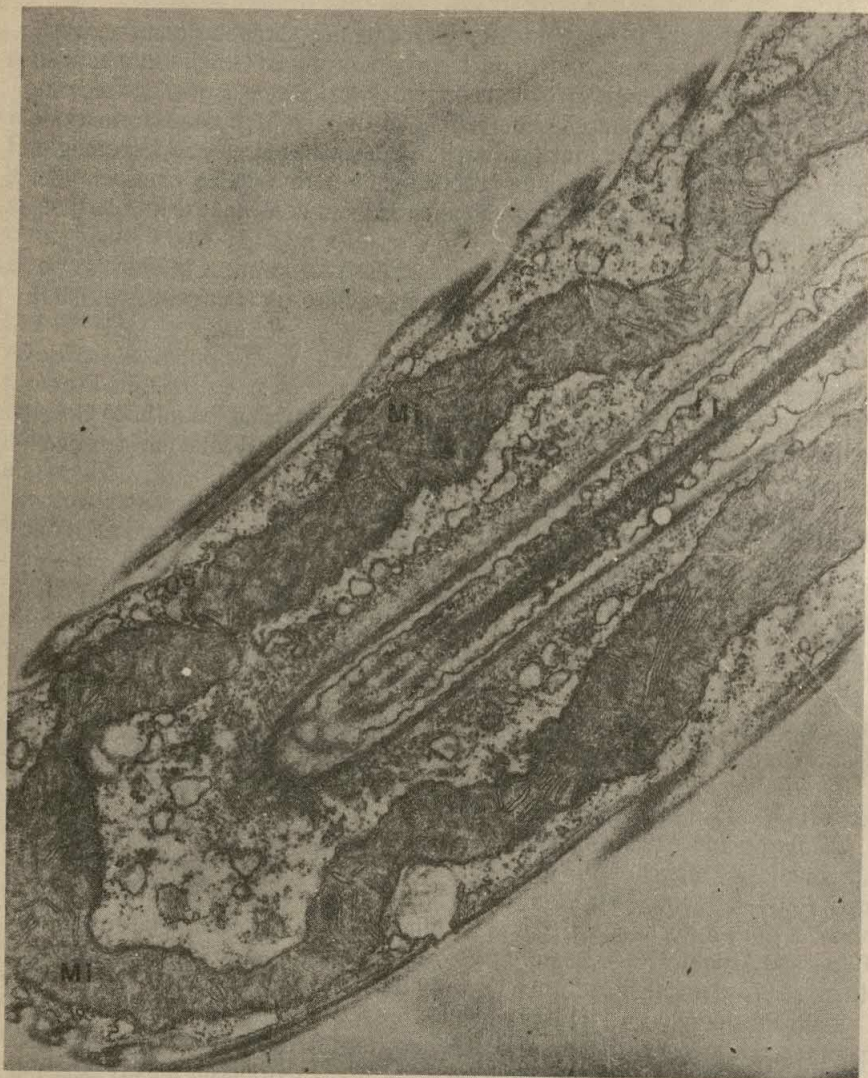


Fig. 5-15 *Euglena gracilis* Z grown in the dark with a giant mitochondrion seen around the gullet region. fl, flagellum; Mi, mitochondrion. ( $\times 18,900$ .) Courtesy M. Lefort-Tran.

Chloromonads have several mitochondria. The mitochondria of *Chattonella subsalsa*, *Gonyostomum semen*, and *Vacuolaria virescens* do not have the complex mitochondrial reticulum which occurs in many other algae (Grobe and Arnold, 1975). However, they do contain abundant tubular cristae as well as a few DNA fibrils (Heywood, 1980).

The mitochondrial DNA of *Chlamydomonas reinhardtii* is quite small ( $4.5 \mu\text{m}$ ) and circular (Ryan *et al.*, 1978), with molecular weight of  $9.8 \times 10^6$  daltons. That of *Euglena gracilis* is about  $16\text{--}45 \times 10^6$  daltons.

Some mitochondria contain ribosomes. Mitochondrially-translated polypep-



tides are coded for by mitochondrial DNA (Ernster and Schatz, 1981). Some mitochondrial genes are now known to have interrupted sequences. One of the most interesting recent discoveries has been that the mitochondrial genetic code has some unique features. Thus, whereas the normal codon assignment for UGA is a stop signal, in mitochondria, this codon signals tryptophan. Similarly, CUN normally codes for leucine but in yeast mitochondria it codes for threonine. However, the coding relations of algal mitochondria have not been worked out so far.

### MICROBODIES (PEROXISOMES AND GLYOXYSOMES)

These are ubiquitous subcellular respiratory organelles in eukaryotic cells. Microbodies also occur in eukaryotic algae (Tolbert, 1972), but their physiological role in cellular metabolism is poorly understood. They seem to lack DNA and energy-coupled electron transfer systems. They are formed by budding from the smooth ER (Tolbert, 1981) to which they sometime remain attached. Microbodies are bounded by single membranes of a composition similar to that of the ER. Certain microbodies which contain catalase and at least one flavin oxidase are called peroxisomes. Others contain one or two additional enzymes, viz., isocitrate lyase and/or malate synthetase; these are called glyoxysomes.

Microbodies range in diameter from 0.1  $\mu\text{m}$  to 1.5  $\mu\text{m}$ . Each is delimited by a single tripartite membrane and contains a finely granular matrix.

The chief function of microbodies is thought to be glyoxylate metabolism (Tolbert and Essner, 1981). They may also confer some protection to the cells against excess of oxygen (Tolbert and Essner, 1981).

Although most algal and green plant peroxisomes contain a glycollate oxidase, certain unicellular green algae and cyanobacteria contain a glycollate dehydrogenase in their chloroplasts or cells. The cyanobacteria lack peroxisomes as well as catalase. According to Tolbert (1981), the virtual absence of peroxisomal enzymes in many unicellular algae, and the development of peroxisomes in mature tissue or in complex cellular associations, are suggestive of the fact that the development of alternate metabolic pathways in peroxisomes is related physiologically to energy balances in the whole tissue. This kind of evidence also indicates that peroxisomes may in some way be vitally associated with higher forms of development rather than being a primitive respiratory organelle.

### EYESPOTS

These organelles occur only in motile cells, zoospores, and flagellated gametes, especially in algae belonging to the Chlorophyceae, Euglenophyceae, Prasinophyceae, Tribophyceae, Chrysophyceae, and Phaeophyceae. Eyespots are rare or absent in the Haptophyceae, Chloromonadophyceae, Dinophyceae, and Cryptophyceae.

In the Chlorophyceae and Prasinophyceae, the eyespot forms part (Fig. 5-16) of a chloroplast but is not obviously associated with the flagella. In the Tribophyceae, Chrysophyceae, and some Phaeophyceae, the eyespot forms part of a chromatophore and is intimately associated with the flagella.

The Euglenophycean eyespots are complex and characteristic structures which are independent of chromatophores but occur adjacent to the flagella. Much work has been done on the eyespots of the green algae and the euglenoids during the past few years. A summary of the more important contributions follows.

Flagellated cells of green algae are typically phototactic (Feinleib, 1980). Most





Fig. 5-16 *Eudorina*, eyespot. (x35,000.) Courtesy K. Ueda.

move toward the light at low light intensities but move away from light when the intensity is high. Those green algae which form both zoospores and flagellated gametes exhibit a differential and opposing type of phototactic response, the gametes generally being positively phototactic and the zoospores generally exhibiting a negative phototaxis. After the gametes have fused, their phototactic response is usually reversed and even the partially fused gamete-pairs tend to move away from the light source (Melkonian, 1980).

Melkonian and coworkers have showed that the phototactic apparatus of green algae consists of two closely-linked structures. The eyespot is part of the chloroplast, is located at some distance from the flagellar apparatus, and consists of up to several layers of closely-packed, carotenoid-containing lipid globules. The second structure includes the eyespot membranes or plasmalemma plus the outer chloroplast envelope membrane overlying the eyespot lipid globules (Melkonian and Robenek, 1980; Robenek and Melkonian, 1981). According to them, eyespot membranes constitute the site of the photoreceptor, whereas the eyespot acts as an auxiliary structure involved in precise phototactic orientation. The green flagellates are believed to use a periodic shading mechanism for light perception in phototaxis. Furthermore, phototactic orientation and photophobic response in green algal flagellates are primarily two separate processes (Bosco and Feinleib, 1979; Nultsch and Häder, 1979).

The eyespot complex of the green algae includes: (1) the eyespot membranes (i.e., the plasmalemma and the outer chloroplast envelope membrane overlying the eyespot) and (2) the eyespot lipid globules. Melkonian (1981) has studied the fate of eyespot lipid globules after zygospore settlement in *Pleurastrum terrestre*. He found that when the flagella are withdrawn, the close association between the outer plastid envelope membrane and the plasmalemma in the eyespot region becomes lost. This is followed by a change in position of the intact eyespot plate (having about 60 globules) inside the chloroplast. While lying peripherally below the chloroplast envelope membranes, the eyespot plate moves around half of the chloroplast to the cell interior where it then rests close to a mitochondrion. Thereafter, the eyespot plate curves around into the interior of the chloroplast and



single eyespot globules enter the pyrenoid matrix of the chloroplast and join with the pyrenoglobules; these latter structures are characteristic constituents of the pyrenoids of this alga (Melkonian, 1981). The eyespot globules are thought to contribute to the lipid store of the growing vegetative cells and probably some transformation of pyrenoglobules into eyespot lipid globules occurs during zoosporogenesis.

In the green algae, the most common form of eyespot contains a single layer of pigmented granules but the eyespots of some species may have 2–9 stacked layers of these pigmented granules. In most green algae, the eyespots are asymmetrically placed, lying in the chloroplast near one side of the cell. The photoreceptor pigment is located in the plasma membrane over the eyespot and such a location provides a means of communication with the flagella, since the plasmalemma is continuous with the flagellar membrane.

In the Volvocales, the layered eyespot contains one internal thylakoid double membrane at the inner face of each lipid droplet layer. The eyespots of most green algae have only a single layer which is formed as a specialization of the chloroplast; this single layer is closely apposed to the plasmalemma at only this point (Foster and Smyth, 1980). Only the plasmalemma appears to contain the photoreceptor pigment.

The green algae in general use the characteristic rhodopsin pigment as a light receptor in phototaxis. This pigment's chromophore is derived as a branch line from the pathway of carotenoid biosynthesis. The euglenoids differ from the above green algal pattern in using a flavoprotein as the photoreceptor pigment. A third kind of pattern found among some algae is best exemplified by *Cryptomonas*. In this, the cells seem to have taken a photosynthetic accessory pigment system present in high concentration and have incorporated some fraction of it into a specialized structure which is used in photoreception. Certain dinoflagellates show this kind of pattern, using a peridinin-like protein for photoreception (Foster and Smyth, 1980).

The eyespot systems of various algae act as a kind of light sensing antenna and are analogous to the radiowave antennas used in radio and television sets. In algal phototaxis, specialised structures independent of the photosynthetic apparatus are used as a kind of directional light wave antennas. An antenna usually includes an eyespot plus certain associated structures. Unicellular motile algae typically move phototactically at about 100  $\mu\text{m}/\text{sec}$ , whereas the larger colonial algae do so typically at around 1000  $\mu\text{m}/\text{sec}$ . This movement is brought about by the beating action of the flagella, and the swimming organisms also frequently rotate rather slowly. As a result of phototactic movement, the organism moves as rapidly as possible into the optimum light zone.

*Euglena* has a saddle-shaped eyespot. It is composed of about 50 dense osmiophilic globules. It acts as a shading device for the paraflagellar body; the latter is the photoreceptor involved in phototaxis. The eyespot is situated on the reservoir opposite the paraflagellar body.

Many workers have isolated and studied the carotenoid pigments contained in the stigmatal globules. As many as 29 compounds have been isolated from light-grown cells of *E. gracilis* by Heelis *et al.* (1979). About 60% of the pigment content of these stigmata is constituted of beta-carotene, diatoxanthin, and diadinoxanthin. Echinenone, canthaxanthin, and cryptoxanthin constitute minor components of the pigments.

Osafune and Schiff (1980) have shown that light and carotenoids profoundly influence the form of the stigma but do not seem to affect the structure of the



paraflagellar swelling. The presence of a normal flagellar swelling is not correlated with the ability to form coloured carotenoids. On the basis of their work on mutants of *E. gracilis*, Osafune and Schiff have concluded that the ability to make eyespot material is correlated with the ability to make coloured carotenoids but light is required to organize this material into the individual spheres characteristic of the normal eyespot (Figs. 5-16, 5-9).

The paraflagellar body is thought to be the light receptor for phototaxis. It contains flavins and flavoproteins which act as photosensitizers. The stigma is located in an outpocketing of the inner surface of the reservoir, opposite the paraflagellar swelling, and not at the base of the reservoir as erroneously believed hitherto (Osafune and Schiff, 1980).

With the exception of dinoflagellates, most other algae, which have eyespots, produce only one type of eyespot in any one group of algae. In dinoflagellates, on the other hand, several different kinds of eyespot are met with. Dodge (1984) reports that the eyespots from five species belonging to the genera *Woloszynskia*, *Peridinium*, and *Glenodinium* fall into three distinct types: independent, eyespot lacking membrane (*W. coronata*); independent, membrane-bound eyespot of a unique type, not present in any other alga (*G. foliaceum*); and eyespot associated with chloroplast (*W. tenuissimum*, *P. cinctum*). In all three cases, the eyespot is located behind the longitudinal groove and there exists a strand of microtubules between the eyespot and the cell covering. This strand probably plays some role in the transmission of directional stimulation from eyespot to flagellum (Dodge, 1984).

### CONTRACTILE VACUOLES

Some motile algae, e.g., unicellular and colonial green algae, euglenoids, and cryptophytes, possess one, two, or more contractile vacuoles which pulsate, shrinking and swelling, and function as osmoregulatory and excretory organelles (Fig. 5-17).

The contractile vacuole complex occupies a fixed position in the cells of cryptophycean flagellates. Hausmann and Patterson (1981) have studied such complexes and found each to comprise the contractile vacuole, a pore, and a vesicular spongione of several 100-nm vesicles. Many of these vesicles have smooth cytoplasmic surfaces, whereas some bear 15-nm coats. These coats superficially resemble a clathrin coat (clathrin is a protein of molecular weight 180,000; it is found in certain localized coatings, with a characteristic polygonal shape, on the cytoplasmic surface of several membranes (see Whyte and Ockelford, 1980)). The membrane width of coated vesicles in different cryptophytes varies from 6.5 nm to 7.1 nm, whereas that of smooth vesicles varies from 6.2 nm to 7.8 nm. The vesicle diameters in the case of coated vesicles range from about 46–143 nm, whereas the same for smooth vesicles range from 42–205 (Hausmann and Patterson, 1981). The vesicles of *Rhodomonas* spp. are somewhat smaller than those of *Chilomonas paramecium*. Both coated and smooth vesicles can occur simultaneously in a cell. According to Hausmann and Patterson, smooth vesicles aid the segregation of fluid from the cytoplasm and are also involved in the filling up of the vacuole. Coated vesicles exist only as independent vesicles and as coated pits in the contractile vacuolar membrane. Whereas smooth vesicles are formed by vesicle-vesicle fusions, no such fusions occur during the formation of coated vesicles. Coated vesicles may be involved in retrieving certain specific membrane components from the contractile vacuole (Hausmann and Patterson, 1981).

In many Chrysophyceae, the position of the contractile vacuoles is not a



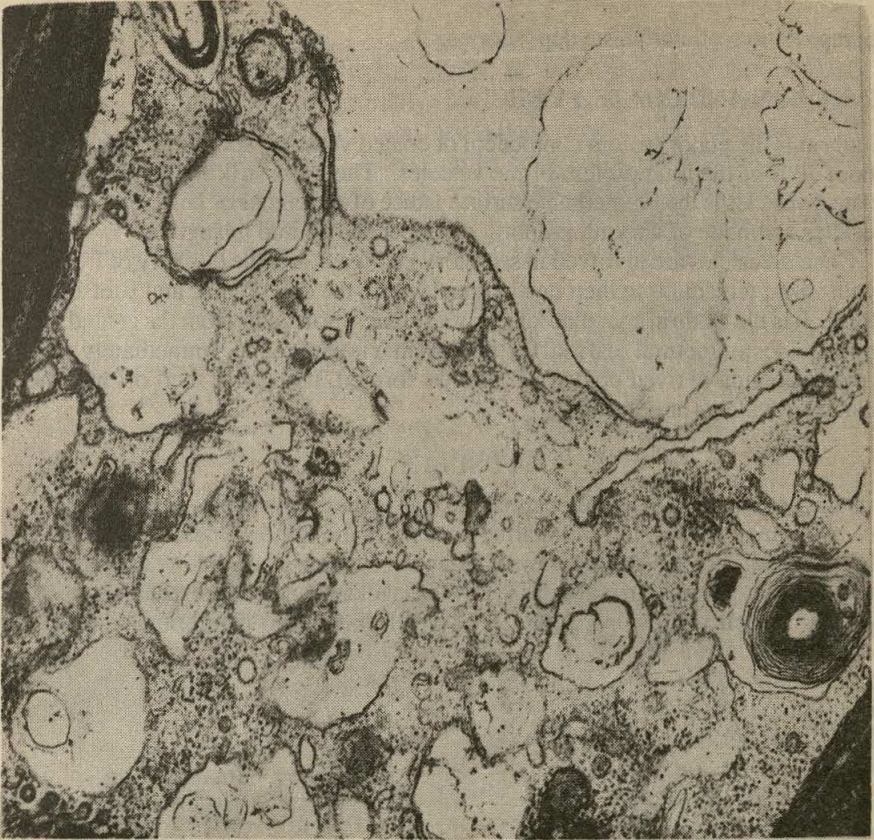


Fig. 5-17 *Euglena*. Contractile vacuole, coated vesicles, and other large vesicles. (x23,050.) Courtesy K. Ueda.

taxonomically important feature. Usually they are anterior in cells with an *Ochromonas* type of organization and posterior in those with a *Mallomonas* type of organization. But there are several exceptions to this generalization, e.g., some species of *Ochromonas* have posterior contractile vacuoles, whereas some of *Mallomonas* have their contractile vacuoles located anteriorly (Skuja, 1948). *Chrysosphaerella brevispina* has two posterior contractile vacuoles as well as a large chrysolaminarin vacuole (Preisig and Hibberd, 1983). This alga also contains certain vesicles (muciferous bodies) with osmiophilic contents near the flagellar pit. A few other vesicles with fibrillogranular contents occur just beneath the plasma membrane (Preisig and Hibberd, 1983). Probably, scales or groups of individuals may be held in position by the discharged contents of these muciferous bodies.

Freshwater Chloromonadophyceae have conspicuous contractile vacuoles which swell to about 10  $\mu\text{m}$  in diameter just before discharging. According to Heywood (1978), the contractile vacuole of *Vacuolaria virescens* is a permanent structure, the fine structure of whose membranes is quite distinct from that of the other cell membranes.

Cells of many symbiotic and free-living dinoflagellates contain accumulation bodies. These structures sometime resemble the food vacuoles in such phagotrophic algae as *Ceratium hirundinella*. Taylor (1968) suggested that these bodies



represent intracellular waste depositories.

## CLATHRIN AND COATED VESICLES

Clathrin is the major protein component of coated vesicles, sometime making up about 70% of the total protein of the vesicles. The most striking feature of the coated vesicles is their remarkable surface lattice of pentagons or hexagons. Some vesicles are made of varying proportions of pentagons and hexagons.

Coated vesicles are involved in secretory pathways and the delivery of freshly synthesized molecules to their destinations within the cell (Pearse and Bretscher, 1981). It is the clathrin coat that selects or excludes molecules from the coated pit, and provides a structural scaffold for the vesicular invagination. Immediately after a plasmalemma-derived coated vesicle has formed, its coat is shed off and the vesicular contents are discharged.

## PUSULES

Pusules are vacuoles of a more permanent nature than contractile vacuoles. They occur in many freshwater and marine species of the Dinophyceae. Their chief function seems to be osmoregulation. Each pusule consists of an invagination of the plasma membrane from the base of the flagellar canal, adpressed to a membrane of the cell vacuolar reticulum. The pusule has a two-membrane thick wall

which surrounds a vesicle opening to the outside of the cell. Dodge (1972) has classified the pusules into the following main types: (1) those with vesicles opening directly into the flagellar canal, e.g., *Gymnodinium nelsonii*; (2) those with vesicles opening into a pusule-collecting chamber; the chamber arises from the flagellar canal, e.g., *Amphidinium herdmanni*; (3) those with vesicles opening into a part of a sinuous tube whose distal end is internally lined with fine, elongated, hairy projections, e.g., *Woloszynskia coronata*; (4) those devoid of vesicles and entirely made of two closely appressed membranes, e.g., *Glenodinium foliaceum*; and (5) those consisting of a much convoluted sac-like structure having numerous invaginations, e.g., *Prorocentrum* sp.

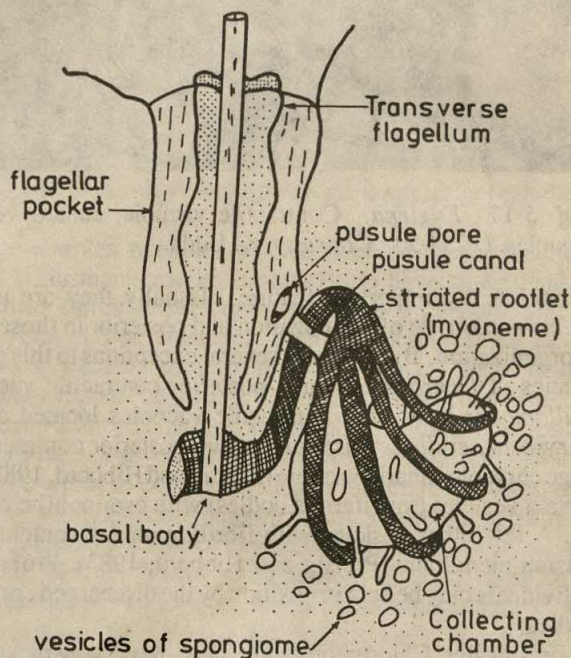


Fig. 5-18 The structure of a pusule (after Cachon *et al.*, 1983).



Both *Woloszynskia coronata* and *Amphidinium cryophilum* have complex tubular pusules with vesicles. These pusules lie in a vesicular sheath and consist of 300-nm tubules which are partially connected with many small vesicles (*W. coronata*) or branches (*A. cryophilum*) (see Wilcox *et al.*, 1982). The pusule of *W. coronata* is composed of rough tubules bearing several small papillae on their inner surface; such papillae are lacking in *A. cryophilum* (Wilcox *et al.*, 1982).

The pusules of dinoflagellates appear to be under the control of large bundles of 2.4 nm non-actin filaments that correspond to the striated rootlets of their two flagella (Cachon *et al.*, 1983).

Whereas the chief function of pusules in freshwater dinoflagellates is osmoregulation, in marine species they seem to play an excretory role (Fig. 5-18).

How do pusules differ from contractile vacuoles? In the latter, osmoregulation occurs as a result of active pumping of intracellular solutes. The pump fills and swells up the radial canals whose contents are then discharged into the collecting chamber, which in turn distends until the pore opens, expelling the contents. In contrast, in the pusule (Fig. 5-19), the pore always remains open. The solutes from the collecting chamber flow continuously, as in the case of many freshwater in-



Fig. 5-19 *Amphidinium cryophilum*. Section of the highly branched portion of the pusule with both 300-nm and 800-nm tubules. The area is seen to be extensively vesiculated. (x33,750.) Courtesy L.W. Wilcox.



vertebrates. No periodical swellings of the radial canals are observable (Cachon *et al.*, 1983).

### EJECTILE ORGANELLES

Some unicellular motile algae possess ejectile organelles of unknown function and mode of action. In response to such stimuli as contact, heat, or chemicals, these organelles discharge some tube- or thread-like structure from the cell surface.

Many dinoflagellates, cryptomonads, chrysophytes, *Pyramimonas*, and *Entosiphon* (Euglenophyceae) possess ejectile organelles. In dinoflagellates such as *Gymnodinium* and *Oxyrrhis*, these organelles are elongated, fusiform structures consisting of a narrow neck and a wider shaft (2–4  $\mu\text{m}$  long) and are attached to the thecal membranes of the cell by means of the neck (Dodge and Crawford, 1971; Hibberd, 1970). The neck harbours twisted fibres which are connected (Figs. 5-20, 5-21) at their proximal end with the main body of the organelle. When stimulated, these organelles release long, solid, banded threads which are squarish or rhombic in transection.

The ejectile organelles of cryptomonads consist of straight tubes with bent or crooked tips. When fully discharged, such organelles become completely detached from the cell. Most of their internal space is occupied by a reel of spirally coiled membranous material which surrounds a narrow channel and a V-shaped depression. A second small reel of membranous material is occasionally found in the V-shaped depression and is connected to the larger reel by means of fibrous connections. During discharge of the organelle, these reels shoot out and unwind to form a hollow tube. In the Dinophyceae and Chrysophyceae, it has been shown that the ejectile organelles arise from Golgi vesicles.

All chloromonads have trichocysts or mucocysts. These organelles are membrane-limited structures about 2.5  $\mu\text{m}$  long and 0.5  $\mu\text{m}$  in diameter. They are made of tightly packed fibrils originating from the Golgi apparatus (Heywood, 1980). Discharged trichocysts extend from the cell as long, mucilaginous processes.

### FIBROUS VACUOLE ASSOCIATED ORGANELLE

This is a discrete subcellular organelle unique to the red algae.

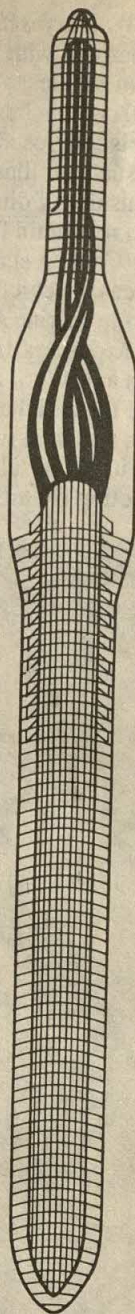


Fig. 5-20 Sketch of longitudinal section through an undischarged trichocyst of *Gonyaulax polyedra* (after Bouck, 1966).



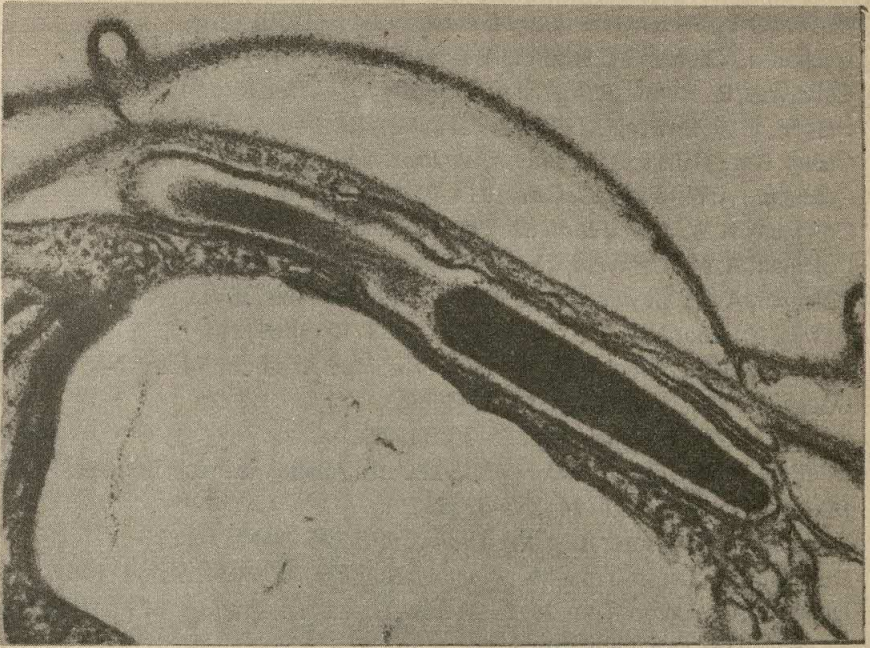


Fig. 5-21 *Amphidinium cryophilum*. Electron micrograph of trichocyst just beneath the amphiesma. (x58,000.) Courtesy L.W. Wilcox.

An excitement in phycological circles was created when Simon-Bichard-Breud (1971, 1972) announced the presence of a non-emergent flagellum in the spermatangium of *Bonnemaisonia hamifera*, a red alga. Subsequent studies have revealed that the so-called non-emergent flagellum (appareil cinétique) is in fact not a flagellum but a fibrous vacuole associated organelle involved in early stages of fibrous vacuole formation (Broadwater and Scott, 1983). Fibrous vacuole associated organelles are now known to be present in some other red algae also, e.g., *Polysiphonia harveyi* and *Dasya* sp. They arise from endoplasmic reticulum and initiate formation of the fibrous vacuoles characteristic of the Florideophyceae spermatangia and spores.

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## 6 Cell Membranes and Cell Walls

### PLASMA MEMBRANES

A 7–8 nm thick plasmalemma surrounds the protoplast of most algal cells and, in some cases, e.g., *Dunaliella* and *Ochromonas*, this is the only covering around the cell. In the vast majority of algal cells, a cell wall is secreted outside the plasma membrane.

The gametes of some algae, e.g., *Chlamydomonas reinhardtii*, are bounded only by the plasma membrane. During gametic fusion in this species, a distinctive fertilization tube is formed; the plasmalemma of this tube possesses numerous small projections (up to 0.5  $\mu\text{m}$  long) (Friedmann *et al.*, 1968). The plasmalemmas of the two mating gametes fuse together at their point of contact.

In many algae, e.g., *Scenedesmus*, groups of microtubules are found lying very close to the plasmalemma.

In *Klebsormidium*, the plasmalemma is lined by transversely oriented wall microtubules.

Biological membranes contain a lipid bilayer. Their protein: lipid: carbohydrate ratios vary greatly from membrane to membrane. The lipids are mainly phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, sphingomyelin, and cholesterol. Some membranes are rich in glycolipids (*vide infra*).

Membranes contain two kinds of proteins, viz., peripheral and integral. The latter are hydrophobically bonded and can be extracted only after detergent treatment, whereas the former are loosely-bound and are easily removed by ionic manipulations. Some proteins are confined to one side (outer or inner) of the bilayer, and some penetrate it partly or completely. The major part of the integral protein in most membranes is in the polar regions of the bilayer (Robertson, 1981).

The plasma membrane constitutes a selectively permeable barrier that regulates the relationship between the cell's internal activities and the external environment. It is a lipid-protein-carbohydrate structure. Its selective permeability to ions makes it possible for a potential difference to be built up between the cytoplasmic and external sides (De Loof, 1986). In certain conditions, a cell can drive an electrical current, carried by ions, through itself.

Dolowy (1984) has reviewed the structure and function of cell membranes with special reference to the bioelectrochemistry of cell surfaces. Singer and Nicholson (1972) proposed a model of the cell membrane according to which the membrane is a lipid bilayer in which active protein molecules are embedded by their hydrophobic parts (Fig. 6-1). Most cell membranes have more protein than lipid. The membrane proteins are of two classes: (1) the intrinsic proteins, embedded in the lipid layer; and (2) the extrinsic proteins, which are merely adsorbed at the cell surface. Some protein structures such as ion pumps and channels (Fig. 6-1) appear to span the membrane.

In recent years, the fluidity of the plasma membrane, and the asymmetrical distribution of ion pumps and channels have attracted more attention than the basic structure and function of membranes. Cells can build up a potential difference of



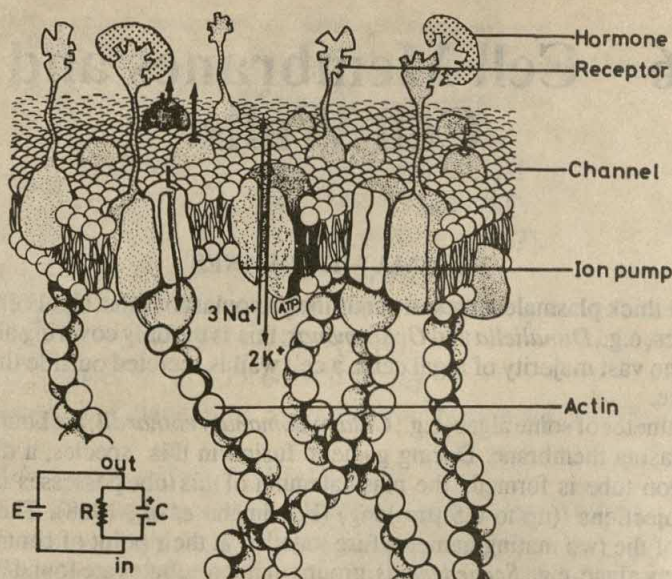


Fig. 6-1 Sketch of the structure of the plasma membrane showing the location of ion pumps and channels. A  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase and an ion channel which opens when a hormone binds to its receptor are also shown. *Inset*: The membrane behaves electrically as a resistance ( $R$ )-capacitance ( $C$ ) network, shunted in parallel.  $E$  is the potential difference over the membrane. (After De Loof, 1986.)

up to about 200 mV over their plasma membrane, and this has important consequences in cell functioning. It appears that the asymmetrical distribution of ion pump/channel activity in the plasma membrane and the cell's ability to drive ionic/electric current through itself, are closely linked properties.

In recent years, the internal structure of several biological membranes has been examined very clearly by means of freeze fracture electron microscopy. Biomembranes contain special arrays of intramembrane particles at certain regions (Satir, 1980). These intramembrane particles represent discontinuities in the fracture plane through the lipid matrix, mostly or entirely by integral membrane proteins or glycoproteins (Verkleij and Ververgaert, 1978). These particles are clearly associated with proteins in membranes. These arrays generally consist of integral membrane particles and are generally stable. This stability of intramembrane particle arrays is believed to arise from their interactions with microtubules, microfilaments, or other similar components (Satir, 1980). Weiss *et al.* (1977) and Robenek and Melkonian (1981) reported the presence of intramembrane particle arrays in the plasmalemma of *Chlamydomonas reinhardtii*. Some of these arrays are located near the flagellar base, whereas others occur remote from flagellar bases. One type of arrays, called induced arrays, interact with cytoplasmic components. Another type, designated intrinsic arrays, represents a discrete class of proteins which are built into the biomembrane and these assemble spontaneously to form the particle arrays.

Robenek and Melkonian (1981) have made a freeze fracture study of the plasma membrane of *C. reinhardtii*. They found that the intramembrane particle arrays in this alga are deficient in 3-beta-hydroxysterols and may be regarded as special lipid arrays. Biomembranes usually exhibit some lipid asymmetry (Rothman, 1980).



Sometime the outer and inner halves of a membrane show a similar asymmetry in respect of sterol content (Fisher, 1976). The work of Robenek and Melkonian (1981) on the planar distribution of 3-beta-hydroxysterols in the plasmalemma of *C. reinhardtii* has shown that virtually no sterols occur in areas characterized by intramembrane particle arrays. Robenek and Melkonian are of the opinion that the absence of such sterols from the arrays of intramembrane particles is a prerequisite for the active functioning of the particle arrays.

Several workers have reported the association of changes in the plasma membrane structure with alterations in cellular behaviour and plasma membrane functions (DeLaat *et al.*, 1981) but such findings have not yet been reported for algae. A layer of parallel filaments located beneath the cell membrane of some Leptodiscinae (marine dinoflagellates) plays an important role in cellular contraction. Such contraction occurs suddenly when the ambient water is disturbed. These filaments are 2.5–3 nm in diameter and are probably non-actin; they are helically-coiled and doubly twisted forming tubular structures upon contraction (Cachon and Cachon, 1984). Similar non-actin contractile organelles are also present in several other groups of Protists.

The plasma membrane often contains indented sites with a characteristic bristly cytoplasmic surface, forming coated pits. These pits bud into the cytoplasm, forming coated vesicles. These coated pits and vesicles seem to act as molecular sieves that permit certain proteins to pass through the membrane but obstruct the passage of others (Pearse and Bretscher, 1981). The proteins which are selected to enter a coated pit then bind to clathrin, the major scaffolding protein on the cytoplasmic surface of the coated vesicles.

Recent researches have established the involvement of coated vesicles in the transfer of newly synthesized proteins from the endoplasmic reticulum to the Golgi apparatus and then to the plasmalemma, in a highly specific manner. The coated vesicles seem to constitute an important device by which membrane proteins are sorted out to move to their different destinations.

Coated vesicles may not only be derived from the plasmalemma but also from the endoplasmic reticulum and the Golgi apparatus. Their function is to pinch off vesicles into the cell cytoplasm. Those vesicles which bud off from the endoplasmic reticulum transfer membrane fragments to the Golgi apparatus and elsewhere within the cell. These transactions accompany a net transfer of lipid away from the ER to the Golgi, plasmalemma, and other organelles (Pearse and Bretscher, 1981).

Osmotic factors play an important role in algae since membrane ion pumps are hydrostatically regulated. Hastings and Gutknecht (1974) studied this problem in *Valonia macrophysa* and found that a negative feedback loop is used to regulate the turgor pressure in this alga which maintains a 1.5 atmosphere turgor pressure by holding the osmolarity of its intracellular sap somewhat above the osmolarity of the external sea water. The vacuolar sap contains  $K^+$ ,  $Na^+$ , and  $Cl^-$  ions which are osmotically active.

The plasma membrane of the brown alga *Sphacelaria tribuloides* along the cell wall forms finger-like infoldings which are associated with mitochondria (Fig. 6-2). Figure 6-3 shows the infoldings at higher magnification.

### PERIPLAST

The cell covering in the Cryptophyceae is more complex than a simple plasma membrane and is called periplast. It consists of a continuous outer membrane on



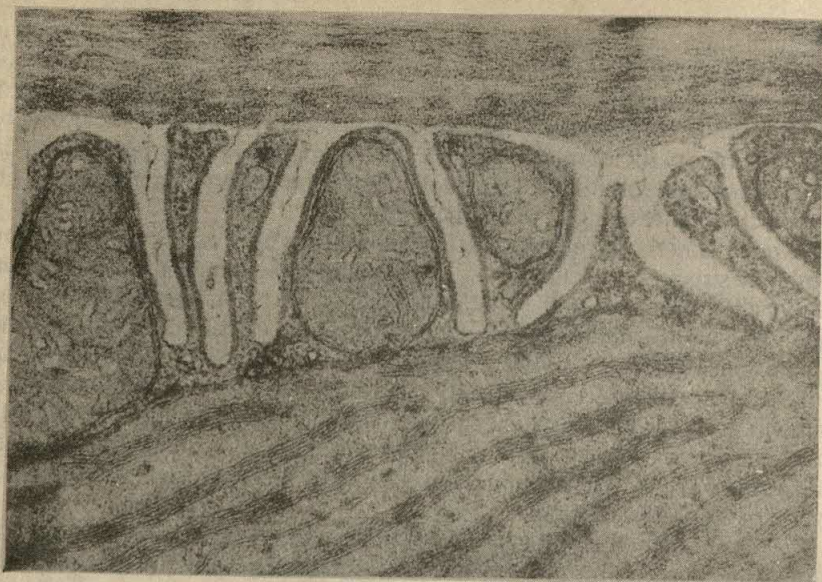


Fig. 6-2 Electron micrograph of section through part of a vegetative cell of *Sphacelaria tribuloides* seen in longitudinal section. The plasmalemma along the walls forms finger-like infoldings showing associations with mitochondria. (x11,000.) Courtesy B. Galatis.

the outer side of which another outer layer may sometime be present. The middle layer of the periplast is the plasma membrane. On the outer side of this membrane lies a fibrous layer, whereas on the inner side of the plasma membrane is a layer of proteinaceous plates. The periplast of *Chroomonas* sp. is about 250 Å thick. It consists of a plasma membrane that is continuous over the whole cell. The periplast is made up of regularly aligned plate-like areas. These areas are delineated by shallow ridges extending along the antero-posterior axis of the cell and also by certain lateral grooves (Gantt, 1971). Small ejectile organelles, called trichocysts, occur as bumps or papillae at the intersections of the antero-posterior ridges and the lateral grooves, and are located posterior to the lateral grooves. These trichocysts seem to form part of the periplast. In most cryptophytes, two trichocysts are located in the corners beneath the anterior facing edges of each proteinaceous plate of the periplast. These trichocysts protrude outward (in *Hemiselmis* they do not protrude outward).

The proteinaceous plates are variously arranged in different species. *Hemiselmis* spp. and *Chroomonas mesostigmatica* form longitudinally offset rows of rectangular plates. In *Cryptomonas*, the plate areas are arranged in hexagonal or polygonal pattern.

The periplast of euglenoids is also sometime called pellicle. It is made of numerous flat, articulating, proteinaceous segments (Schwelitz *et al.*, 1970) disposed spirally around the whole cell and covered by means of an outer plasmalemma. These interlocking strips fuse into one another at each end of the cell. The periplastic strips of *Euglenaspirogyra* have continuous ridges which face outward and which articulate in a groove along the overlapping edge of the adjacent strip. Some sideways movement can occur between two adjoining strips





Fig. 6-3 The same as Fig. 6-2, but at higher magnification. The infoldings form cytoplasmic pockets which are occupied by mitochondria. (x49,000.) Courtesy B. Galatis.

by the sliding action of the ridge in the groove and by stretching and compression of periplastic material.

Dinoflagellates may or may not form a cellulose cell wall. Some lack a cell wall, others have only a thin cell wall, and still others are characteristically armoured or thecate. The thecate members have their cell covering or theca composed of numerous articulated and variously-sculptured plates. The theca is made up of microtubules and flattened vesicles. These vesicles may be empty or may contain delicate polygonal plates, or they may enclose cellulosic plates.

The naked alga *Gymnodinium splendens* has its external surface covered with rounded projections anchored by numerous fine filaments (Herman and Sweeney, 1977).

The cell covering (called amphiesma) of dinoflagellates consists of an outer membrane, flattened vesicles (which enclose cellulosic plates in some species), and a pellicle beneath which lies a membrane bounding the cytoplasm (Loeblich, 1970). In a survey of many dinoflagellates, Morrill and Loeblich (1981) detected the presence of a pellicular layer in 15 out of the 20 genera studied. Most organisms which formed pellicles were capable of reinforcing this layer with cellulose. Although pellicle is widespread among dinoflagellates, its composition is not uniform. The pellicles of some dinoflagellates, e.g., *Peridinium* and *Heterocapsa*, are acetolysis-resistant, whereas those of some others, e.g., *Gonyaulax*, are not.



## SCALES AND COCCOLITHS

One common character shared by many chrysophytes is the presence of characteristic scales outside of the plasma membrane. These minute scales may be silicified as in *Synura* and *Mallomonas* or non-silicified as in *Chromulina placentula* and *Sphaleromantis tetragona*. Some chrysophytes, often placed in the class Haptophyceae, have their cells covered with scales of an organic matter but these are comparatively less varied than those found in the Chrysophyceae. The scales of the Haptophyceae are more akin to those of the Prasinophyceae. In the latter, the motile cell is covered with one to several characteristic layers of organic scales. Some prasinophytes tend to have a flexible non-cellulosic theca in place of the scales. *Dolichomastix* has a simple periplast made up of monomorphic, rimless plate-scales which resemble the similar scales found on its flagella (Manton, 1977).

Certain members of the Haptophyceae produce elaborate and conspicuous outer scales called coccoliths (Fig. 6-4), which can be seen even under the light microscope. These coccoliths are made up of calcite (calcium carbonate). Diverse coccolithophorids have variously patterned and ornamented coccoliths.

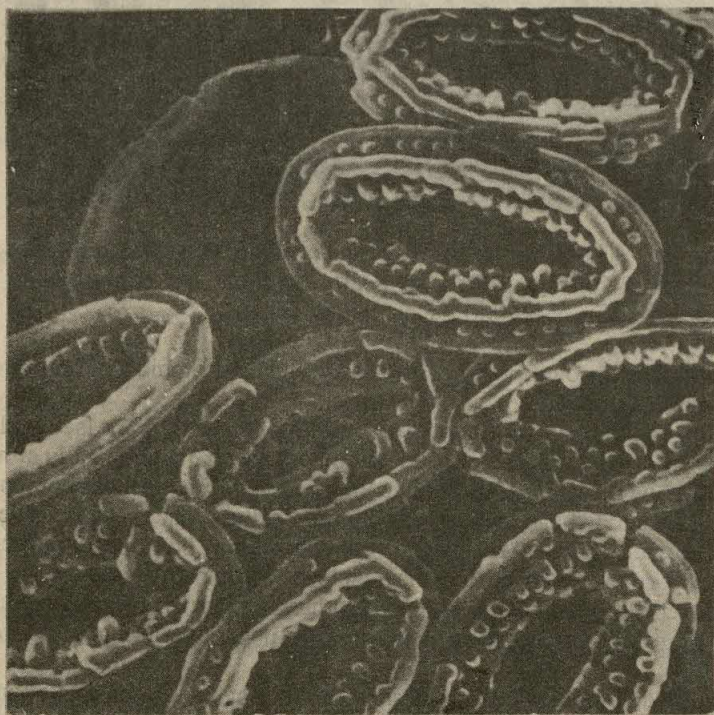


Fig. 6-4 Scanning electron micrograph of *Cricosphaera roscoffensis*, showing coccoliths. (x20,000.) Courtesy P. Gayral.

Outka and Williams (1971) have described the various stages in coccolith formation in *Hymenomonas*. Formation of scales and parts of coccoliths begins in the cisternae of the conspicuous and large Golgi body, whereas stages in coccolith assembly are seen in certain vesicles which become detached from the Golgi body. The assembly process involves the following stages: (1) the forma-



tion of delicate oval base plates made of organic material and patterned with spokes radiating from a line passing through the long axis of the plate, (2) the formation, around the developing base plates, of large numbers of small vesicles, each of which contains coccolithosomes (each coccolithosome is composed of about a dozen granules of about 6–8 nm), (3) the release of the coccolithosomes into the base vacuole followed by their association with the rim of the plate and gradual formation of an outline of the anvil-shaped subunits which finally make up the rim (the coccolithosomes are used up during the process of coccolith development, presumably as precursors for the material of the rim), and finally (4) the assumption by the coccolith rim of its typical shape and its calcification as a result of crystallization of calcium carbonate in the matrix of the rim (Outka and Williams, 1971).

Out of about half-a-dozen coccolithophorids studied under the electron microscope until recently, only the motile stages of *Coccolithus pelagicus* show extracellular mineralization of the organic matrix scale; the others, e.g., *C. huxleyi* and *Hymenomonas* spp., show intranuclear mineralization (Pienaar, 1969). Pienaar (1971) reported that *Hymenomonas carterae* produces its delicately sculptured coccolith plates on an organic base plate within the Golgi cisternae.

Glider and Rosowski (1976) made a detailed study of the scales, bristles, and spines of *Mallomonas*. This alga bears species-specific siliceous scales. *M. lychenensis* bears anterior circular bristle-bearing scales and rounded rhomboidal body scales, and its posterior scales have spines. All scale surfaces have rows of uniformly-shaped papillae and rows of depressions which contain smaller pores. *M. caudata* produces ovoid to elliptical scales; its bristles are produced from flat siliceous sheets which fold to form hollow, serrated tubes. Three types of scale are produced in *M. tonsurata*, viz., (1) anterior, bristly scales consisting of a dome, shield, and flange, (2) domeless body scales, and (3) domeless posterior scales which bear a short spine.

The scales of the marine alga *Paraphysomonas foraminifera* are perforated by many pores. Scales of this species as also those of *P. imperforata* have a central dome, bearing a long spine, and a shallow annular fold with a radius one-half of that of the scale. A wide variety of scale types occurs in different species of this genus. These include flat plate-scales with an open meshwork structure (*P. homolepis*), dish- or bowl-shaped scales (*P. punctata*), flat-bottomed dish-shaped scales (*P. runcinifera*), undulate scales with a crater (*P. undulata*), and crown-shaped scales (*P. diademifera*, *P. morchella*) (Preisig and Hibberd, 1982). *P. cycliphora* has scales with a solid flat base which bears a small meshed cup-shaped projection distally.

In the Synuraceae, the siliceous scales and bristles are produced in vesicles from the chloroplast ER. They then deposit on the cell surface (Wujek and Kristiansen, 1978). Silicon requirements are just as in diatoms, and growth is inhibited by germanium dioxide (Lee, 1978; Hibberd, 1979).

The species *Chrysochromulina pringsheimii* has an elaborate cocoon-like scaly casing from which the monad can readily disengage itself and crawl away, leaving behind the intact empty case. Four different kinds of scale, viz., large and small plates, and large and small spines, occur in this species. The alga can resort to phagotrophic feeding both in the presence and absence of its scaly covering. The ingestion of solid food by the encased alga seems to be unimpeded by the scales, and also occurs in several other scale-bearing chrysophytes.

Some coccolithophorids, e.g., *Syracosphaera*, have two layers of different coccoliths, a phenomenon called "dithecatism" (Klaveness and Paasche, 1979).



In rare cases, coccoliths of two different species or genera may be found on a single cell.

A few dinoflagellates such as *Oxyrrhis marina*, *Heterocapsa triquetra*, and *Glenodinium* sp. possess body scales. These generally occur external to the thecal plates (Pennick and Clarke, 1977).

Prasinophycean flagellates characteristically form non-mineralized organic scales which cover their cell as well as flagella. The cells of *Pyramimonas* commonly have three kinds of scale, viz., underlayer, intermediate, and outer body scales (Moestrup and Walne, 1979). The flagella of the same organism are covered with three different kinds of scale called the underlayer, the limulus, and the hair scales. Melkonian and Robenek (1981) have shown that the underlayer scales are squarish and form a continuous layer around the cell body of *P. grossii*, *P. amyliifera*, and *P. parkeae*. These scales are arranged in rows with neither any intervening space nor overlap. The flagella of these species also have a continuous layer of underlayer scales, each flagellum having 24 rows of scales. The detailed morphology of the underlayer scales of the cells of the three species differs, some having thickened rims, 9 nm high and 16 nm wide. In contrast, the flagellar underlayer scales of the three species are indistinguishable, all being pentagonal. Table X compares different species of *Pyramimonas* with reference to scale types.

**Table X** Scale types in selected species of *Pyramimonas* (after Pennick, 1984)\*

Species	Body scales		Flagellar scales
	Outer layer	Inner layer	Outer layer
<i>obovata</i>	3	Cover whole body surface	Have longitudinal striations
<i>occidentalis</i>	1	Confined to flagellar tip	Have unchannelled lamellae
<i>disomata</i>	1	Confined to flagellar tip	Have longitudinal striations
<i>gelidicola</i>	1	With footprint scales	Have numerous pores
<i>spinifera</i>	1	Confined to flagellar pit	Have transverse striations
<i>cirolanae</i> **	2	Cover whole body surface	Have numerous pores
<i>grossii</i> **	1	Cover whole body surface	Have unchannelled lamellae
<i>tetrahynchus</i>	2	Cover whole body surface	Have numerous pores

\*All species have middle layer body scales.

\*\*Also have trichocysts.

According to Melkonian and Robenek (1981), the underlayer scales are the morphologically most conservative scale type, then followed by the intermediate body scales, whereas the outermost scale type shows the greatest structural variability.

The conservative underlayer body scales of the *Pyramimonas* type also occur in many other genera of the Prasinophyceae, e.g., *Nephroselmis* and *Tetraselmis* (Norris, 1980). Stewart and Mattox (1978) speculated that the cell wall of their class Chlorophyceae might have evolved from fusion of body scales in a motile, prasinophyte-type ancestral flagellate. Ultrastructural studies of reproductive cells of the Ulvophyceae and Charophyceae have shown that these cells have body scales very similar to the underlayer scales of *Pyramimonas*. The phylogen-



etic implication of this observation is that these scales may not have contributed to the cell wall evolution in the above three classes of green algae. Thus, the squarish type of underlayer scales of *Pyramimonas* have not been seen in any Chlorophycean alga; they may either have been lost during the course of the Chlorophycean evolution or may have fused to form a primitive cell wall or theca that later became transformed into the typical Chlorophycean cell wall (Melkonian and Robenek, 1981).

Many dinoflagellates have an anteriorly-situated apical pore in their thecal covering. Three types of thecal plate, viz., pore plate, cover plate, and canal plate, are involved in the organization of the apical pore in marine dinoflagellates (Dodge and Hermes, 1981). The apical pores of *Helgolandinium* and *Pyrophacus* are slit-like and have only the pore plates. A hook-shaped pore and a simple cover plate characterize some species of *Gonyaulax*, whereas other species have tubular pore lacking cover plate (Fig. 6-5). In *Protopteridinium* and *Scrippsiella*, the pore is tubular and a canal plate is present. In *Pyrodinium*, there is no canal plate but an elaborate cover plate is present; the shape and ornamentation of this cover plate vary widely in different members of the genus (Dodge and Hermes, 1981). All three plates (pore, cover, and canal) are present in *Heterocapsa triquetra* and *Glenodinium hallii*.

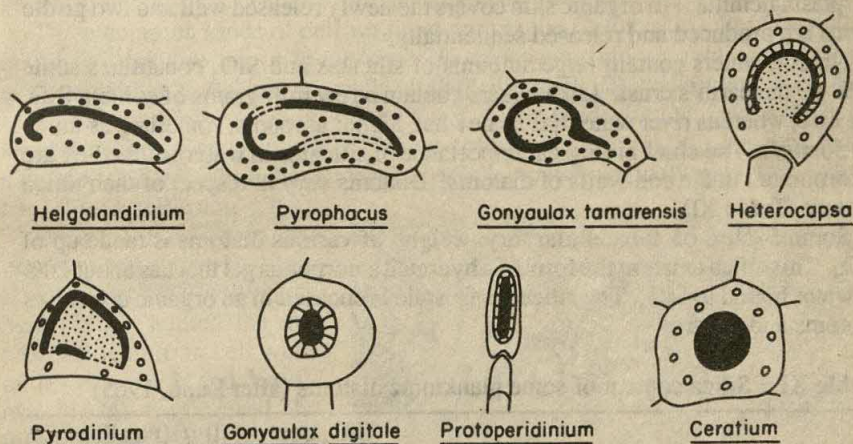


Fig. 6-5 Diagrammatic illustration of some important types of apical pore structure met with in marine Dinophyceae (after Dodge and Hermes, 1981).

In *Ceratium*, the pore plates are polygonal but there are no cover plates or canal plates. The apical, circular pore is commonly located at the distal end of a long apical horn which is formed from extensions of the four apical plates.

The different types of apical pore met with in dinoflagellates are diagrammatically shown in Fig. 6-5.

## FRUSTULES

Just as  $\text{CaCO}_3$  plays an indispensable role in the formation of coccoliths, so does silica in the formation of diatom cell walls or frustules. Frustules of several diatoms have been examined by scanning electron microscopy and other modern microscopic techniques.

Drum and Pankratz (1964), Lauritis *et al.* (1968), Reimann *et al.* (1966), and



others have studied the mode of formation of the silica wall in several diatoms. Silicon deposition vesicles first collect just beneath the plasma membrane at the centre of the site of a new frustule. The vesicles fuse and the membranes form a continuous sac, called silicalemma. The silicalemma later surrounds the area where the new frustule is being synthesized. Silica deposits rapidly and becomes neatly disposed into the characteristic frustule type.

A detailed study of the sequential cell wall development and the biochemistry of silica shell formation in *Navicula pelliculosa* was made by Chiappino and Volcani (1977). In this species, the epitheca and hypotheca are surrounded by girdle bands. Each of the two valves has a central raphe fissure which is bordered by the central raphe rib and interrupted by the central nodule, radiating transapical ribs and a regular series of sieve plates (Reimann *et al.*, 1966). The girdle region possesses 5 girdle bands, of which 3 are on the epitheca and 2 on the hypotheca. Immediately before the start of mitotic division, a third girdle band is added to the hypotheca. Following cytokinesis, the new valve is initiated when a primary central band is formed within a silica deposition vesicle (Chiappino and Volcani, 1977). The band extends along the cell length and has a central nodule. From this nodule, some secondary arms extend and join with extensions of the primary central band and secondary arms, and cross extensions join to form the sieve plate areas. The cell wall seems to be released by a joining of the inner silicalemma and the plasmalemma. An organic skin covers the newly released wall and two girdle bands are produced and released sequentially.

Natural waters contain large amounts of silicates and  $\text{SiO}_2$  constitutes some 50% of the earth's crust. Lake waters contain a few milligrams of soluble  $\text{SiO}_2$  per litre, whereas river water has somewhat higher amounts, sometime as much as 50 mg/l. The chief biological importance of silicates in water is that they are incorporated in the cell walls of diatoms. Diatoms vary in respect of their silica content (Table XI).

Some 5–60% of the cellular dry weight of various diatoms is made up of  $\text{SiO}_2$ . This silica exists in the form of a hydrated amorphous gel that has about 10% of water bound to  $\text{SiO}_2$ . The siliceous frustule is encased in an organic coating of proteins and sugars.

**Table XI** Silica content of some planktonic diatoms (after Lund, 1965)

Diatom	$\text{SiO}_2$ content ( $\mu\text{g}/10^6$ cells)	
	Range	Mean
<i>Synedra acus</i>	1100–1250	1175
<i>Cyclotella socialis</i>	850–1150	1000
<i>Tabellaria flocculosa</i>	250–420	350
<i>Melosira italica</i>	165–274	237
<i>Fragilaria crotonensis</i>	170–215	189
<i>Asterionella formosa</i>	43–175	132
<i>Stephanodiscus hantzschii</i>	32–47	41

In *Nitzschia alba*, the silica is deposited within a membrane system, the silicalemma, which develops progressively until the completion of the cell wall (Lauritis *et al.*, 1968). Unlike some other diatoms, e.g., *Navicula pelliculosa* and *Cylindrotheca fusiformis*, in *N. alba* the raphe is deposited last. However, in all these diatoms, the initial silica deposition vesicle is formed in the central



region of the dividing cell. According to Coombs *et al.* (1968), the nucleus, the Golgi apparatus, and microtubules are all involved in the silicification of the cell wall in *Navicula pelliculosa*.

Two types of valvar processes, called labiate and strutted processes, have been reported in some diatoms (Hasle, 1972). Hasle (1973) has shown that the mucilage pore of certain diatoms lacking raphes is the same structure which is called the labiate process in centric diatoms. Monoraphid or biraphid diatoms lack labiate processes and, according to Hasle (1973), the labiate process may be the predecessor of the raphe.

## CELL WALLS

Certain algae, e.g., *Dinobryon*, *Ochromonas*, and *Platymonas*, and certain flagellates form cell walls, coverings, or loricas (Fig. 6-6) which cover the cells incompletely. The lorica or theca of *Platymonas* is formed by the coalescence of numerous tiny stellate particles produced in Golgi vesicles. The loricas of many Chrysophyceae are made up of cellulose microfibrils. They are made of a fibrillar meshwork of various patterns. In *Poteriochromonas*, the fibrils are chitinous rather than cellulosic (Herth *et al.*, 1977).

Complete walls cover the cells of most algae. These complete cell walls are generally composed of a microfibrillar framework plus much amorphous material.

Three common kinds of cell wall met with in the Chlorophyta are: (1) walls mainly of cellulose, e.g., *Cladophora*, *Chaetomorpha*, and *Valonia*; the content of amorphous material in these walls is relatively small; (2) walls with less of cellulose and more of amorphous material, e.g., *Ulva*, *Oedogonium*, *Spirogyra*, and several desmids; and (3) walls lacking cellulose microfibrils, e.g., *Bryopsis*, *Caulerpa*, *Udotea* (these have xylan), *Codium*, *Acetabularia* (mainly have mannan) (Frei and Preston, 1964).

Desmids have been the organisms of choice for studies of green algal cell walls. Mix (1966) studied the microfibril texture in cell walls of desmids. Ueda and Yoshiooka (1976) emphasized the difference between the primary and secondary walls in *Micrasterias americana*. Kiermayer and Dobberstein (1973) showed that the flat vesicles in *M. denticulata* can serve as templates for the formation of wall microfibrils. Whereas most of these studies were done on vegetative cells,

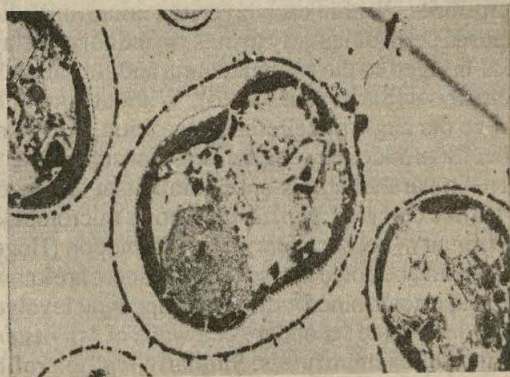


Fig. 6-6 Photomicrograph of cell of *Trachelomonas*, showing lorica. (x2000.) Courtesy K. Ueda.

Noguchi and Ueda (1985) have studied the walls of zygotes. During zygote maturation in *Closterium ehrenbergii*, six wall layers are formed outside the plasma membrane. Of these, layer III is the thickest and is made up of microfibril bundles. The development of this wall layer is accompanied by the production of flat vesicles from dictyosomes. Hexagonal arrays of rosette particles appear in the plasma membrane at the same time, thereby pointing to the possibility that both



flat vesicles and hexagonal particle arrays may be involved in the formation of microfibril bundles. Noguchi and Ueda (1985) further observed that the wall layer VI consists of single microfibrils and that neither flat vesicles nor hexagonal particle arrays can be seen during the formation of this layer.

An unusual kind of cell occurs in *Microspora*. This can be dissociated into distinct segments which are H-shaped in optical section and which contain the end wall and half the outer wall of two adjacent cells. The cell wall of the unbranched filament is made of overlapping H-shaped pieces.

In *Coleochaete orbicularis*, following fertilization, the vegetative cells adjacent to zygotes develop conspicuous, localized, wall ingrowths (Graham and Wilcox, 1983). These ingrowths occur only on those cell walls which are immediately adjacent to zygotes, being absent from other walls of the same cells. According to Graham and Wilcox (1983), the zygotes influence the initiation of wall ingrowth development, and the vegetative covering cells developing the ingrowths strongly resemble the placental transfer cells found in the gametophytes of many higher plants. It seems probable that the covering cells may function in transporting photosynthates to the zygotes. This is akin to the establishment of a nutritional relationship between different phases of the life cycle, similar to that which occurs in embryophytes.

Cell walls of some species of *Pithophora*, but not of *Cladophora*, contain chitin (Pearlmutter and Lembi, 1980).

In most plant cells, microtubules are important in the formation of fibrillar cell walls, but many desmids can synthesize elaborate and complex fibrillar walls without involving microtubules.

There are compelling indications that the orientation in which wall microfibrils are deposited is regulated by cortical microtubules (Gunning and Hardham, 1982). The secondary wall of *Oocystis* consists of about 30 layers of large cellulosic microfibrils. Within each layer, the microfibrils lie parallel to each other, but are disposed perpendicularly to those in the adjacent layers. Cortical microtubules are cross-bridged to the plasmalemma and lie parallel to the innermost layer of wall microfibrils (Sachs *et al.*, 1976). Without microtubules, *Oocystis* cell is unable to change the angle of cellulose deposition, but the microtubules are not necessary either for production of cellulose or for supporting the continued growth of microfibrils in parallel arrays once parallelism has become established.

Unlike *Oocystis*, *Closterium* shows microtubule-microfibril coalignment during primary (not secondary) wall formation (Hogetsu and Shibaoka, 1978).

It should be noted that several instances are known where walls can develop and cell shape determined without any apparent involvement of cortical microtubules. The primary and secondary cell walls of *Micrasterias* and the secondary walls of *Closterium* exemplify this. Similarly, wall microfibrils can be deposited in highly-ordered patterns without participation of microtubules, for instance, in algal scales (Cox, 1980).

Much recent work has centred around the synthesis of the cellulosic wall and the orientation of microfibrils in *Oocystis solitaria* (Robinson and Quader, 1981). The involvement of cortical microtubules in the control of microfibril orientation has been shown. Besides these, two other structures involved in the same control include granule bands and terminal complexes. These two structures collectively interlink the orienting influence of the microtubule and the oriented microfibrils. According to Robinson and Quader, the terminal complexes are immediately responsible for the synthesis of the glucan chains and their assembly into a



microfibril. These complexes are inserted, pair wise and antiparallel to one another, into the outer lipid bilayer of the plasmalemma. The deposition of the crystalline microfibrils pushes apart the terminal complexes in the plane of the outer lipid bilayer. The granule bands determine the direction of movement of the terminal complexes, but their action is subject to modification or regulation by the microtubules also. The granule bands maintain contact with cortical microtubules (these bands are located in the plane of the inner lipid bilayer). Using the freeze fracture technique, Robinson and Quader (1981) studied the changes in morphology of the E-face of plasmalemma following exposure to certain inhibitors of microfibril orientation, synthesis, or crystallization. They found that Congo Red increases the frequency of paired, thickened terminal complexes. Prolonged treatment with this dye causes the insertion of an additional set of parallel terminal complexes in the E-face.

Cell walls of *Pyrocystis* sp. and some other dinoflagellates contain alpha-cellulose arranged in fibrils with crossed parallel texture.

The xanthophyte *Tribonema* has its wall made of overlapping H-pieces and shows randomly arranged cellulose microfibrils. *Vaucheria* cell walls also contain randomly arranged cellulose microfibrils.

Alginic acid is a prominent constituent of the brown algal cell walls. Indeed, quantitatively it is the major polysaccharide of the brown seaweeds. Mannuronic acid residues are the major sugar constituent of many brown algae. These residues are linked through beta-1,4-linkages. In addition, some species have glucuronic acid. The ratio of mannuronic acid to glucuronic acid residues in the alginates of various Phaeophyceae genera may range from less than 0.7 in *Pylaiella*, *Dictyosiphon*, and *Desmarestia* to more than 1.0 as in several Fucales and Laminariales (Percival, 1978). The brown algal walls generally contain a very small percentage of cellulose microfibrils which are randomly arranged. Cell walls of brown algae contain significant amounts of polyuronide alginates and glycans rich in sulphated-L-fucose. The relative proportion of uronic acid and fucose sulphate may vary from species to species (Mian and Percival, 1973; Percival, 1979). *Fucus vesiculosus* seems to lack a discrete fucan sulphate (fucoidan) but developing embryos of *F. distichus* do contain fucoidan.

Some 20–25% of the wall in some red algae is composed of cellulose microfibrils. *Porphyra* lacks cellulose, and its microfibrils are mainly composed of beta-1,3-linked xylan. As much as two-thirds of the dry matter content of some red algae may be made of sulphated polysaccharides. In the Rhodophyta, ester sulphate is usually associated with galactans, whereas in the Phaeophyta it tends to be associated with fucans.

The wall polysaccharide of *Porphyridium* seems to be a glucuronoglucoxylogalactan in which galactose and xylose occur in equal proportions and more than 90% of the galactose is L-galactose. The cell wall also contains about 7.5% of sulphate which may be associated both with galactose and xylose residues (McCandless and Craigie, 1979).

Sometime quite different polysaccharides are produced by different phases of the same species. Thus, whereas the diploid phase of *Chondrus crispus* produces lambda-carrageenan, the haploid phase of the same species produces kappa-carrageenan. In *Porphyra tenera*, whereas the cell walls of the mature thallus are rich in glycine and alanine (Table XII), those of the Conchocelis stage are richer in aspartic acid, glutamic acid, methionine, and certain basic amino acids (Mukai et al., 1981).



**Table XII** Some important differences between cell walls of thallus phase and Conchocelis phase of *Porphyra tenera* (after Mukai *et al.*, 1981)

Parameter	Thallus phase	Conchocelis phase
Total protein content (% dry weight)	15	19-21
Total nitrogen (amino acids; % dry weight)	2-3	3
Histidine (mol %)	Trace	3
Arginine (mol %)	3	7
Aspartic acid (mol %)	6	19
Proline (mol %)	9-10	5
Glycine (mol %)	20	8
Methionine (mol %)	Trace	2
Mannose (mol %)	41	5
Galactose (mol %)	32	77
Glucose (mol %)	11	3

The neutral sugar content of the thallus cell walls of *Porphyra* is more than double that from the Conchocelis; the former mainly contains mannose, whereas the latter is principally galactose (Table XII). Again, whereas the Conchocelis cell wall contains cellulose microfibrils, the mature thallus lacks cellulose but has xylan microfibrils. Thus, the Conchocelis and thallus phases of *P. tenera* differ markedly in respect of their cell wall chemistry.

The biosynthesis of the polysaccharide chains as well as their sulphation are thought to occur in the Golgi bodies (McCandless and Craigie, 1979). The mechanism of deposition of sulphated polysaccharides in algal cells and the functional metabolic significance, if any, of these polysaccharides, is not clear. We also know little about the nature of the covalent linkages in the cell wall though the involvement of certain glycoproteins (*vide infra*) seems likely. In *Porphyridium* and possibly other red algae, xylose residues seem to bind to threonine or serine of the wall proteins but, again, the functional significance of such binding, if any, remains obscure.

### GLYCOPROTEINS (LECTINS)

Lectins are recognition molecules. They have at least one binding site which can bind free or glycosidically-linked carbohydrates (this interaction can be inhibited by some simple sugars). Lectins are not synthesized due to an immune response and lack an immunoglobulin structure. They do not show enzymatic activities towards carbohydrates to which they bind. Simply defined, lectins are sugar binding proteins or glycoproteins of non-immune origin which are devoid of enzymatic activity towards sugars to which they bind and do not require free glycosidic hydroxyl groups on these sugars for their binding.

The past decade has seen a radical change in our ideas about the algal cell walls and the mode of their development. Catt *et al.* (1978) have made a valuable study of glycoproteins from the cell wall of *Chlamydomonas reinhardtii*, with particular reference to the *in vitro* assembly of hydroxyproline-rich glycoproteins which constitute the main structural component of the cell wall of this alga. According to Roberts (1979), the plasma membrane in this alga is covered by a multilayered



cell wall made of hydroxyproline-rich glycoproteins. Only a part of the plasma membrane (that part which covers the two flagella) is directly exposed to the environment, and this particular part has a conspicuous glycocalyx. Since the flagellar surface has a strong affinity for lectins (Katz *et al.*, 1980), the glycocalyx is probably quite rich in carbohydrates, as it is known that the carbohydrate moieties of glycoproteins and glycolipids confer the specificity for various cell-to-cell and cell-substrate interactions (Carter, 1982). One major fraction of these glycoproteins is capable of self-assembly into crystalline structures resembling those present within the intact cell wall. This kind of glycoprotein reassembles into crystalline fragments which attach to the thin inner wall layer, thus making up the completed cell wall.

In this alga, the cell wall is formed unusually rapidly *in vivo*. Cytokinesis is followed immediately by the *de novo* assembly of a completely new cell wall. Catt *et al.* (1978) postulate that the two subunits of the glycoprotein are excreted into the fluid space between the plasmalemma and the mother cell wall, where some self-assembly process leads to the production of the structured cell wall.

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# 7 Flagella

## INTRODUCTION

Except for red algae and prokaryotic classes (cyanobacteria and Prochlorophyceae), most other algae either produce flagellated swimmers or are themselves flagellated in the adult, vegetative phase. For instance, many unicellular and colonial members of the Volvocales are motile. The Ulotrichales and Oedogoniales are not motile but they produce zoospores and gametes which are motile. In the Conjugales (Zygnematales), no flagellated stages occur but their gametes show some amoeboid movement.

The number of flagella per cell and the manner of their attachment have been a useful taxonomic basis since long (see Table IX). Since the advent of electron microscopy, the fine structure of algal flagella has provided an additional parameter of considerable systematic significance. Manton (1965) and Moestrup (1982) have critically reviewed the phyletic implications of flagellar structure in plants and algae.

In recent years, there have been some changes in the terminology of flagella. Originally, heterokont flagella meant that one flagellum in a pair was longer, whereas the other was shorter. Modern usage of this term is quite different, viz., one flagellum is hairy, whereas the other is smooth. Likewise, isokont condition now means that the two flagella of a pair are equal in length as well as appearance, whereas the term anisokont is applied for those genera within isokont groups whose flagella differ in length. However, none of these terms (heterokont, isokont, or anisokont) can be applied to the Cryptophyceae, Dinophyceae, Euglenophyceae, and the order Pavlovales of the Haptophyceae. In these cases, the flagella all represent aberrant types.

The flagella are always associated with an apparatus inside the cell. One flagellum is connected to one basal body and to one or more flagellar roots.

There are two general views regarding the evolutionary origin of flagella and their precursors. Cavalier-Smith (1978) believes that microtubules arose within the nucleus, evolving from the RNA-cores of the prokaryotic chromosome. According to Stewart and Mattox (1980), the flagella came first, with the spindle evolving as an elaboration of the flagellar apparatus.

## EXTERNAL CHARACTERISTICS

A flagellum is bounded by an extension of the plasmalemma. In different classes, there are variations in respect of structures external to this extension of the plasmalemma. Some Chlorophyceae and Haptophyceae produce flagella which are quite smooth, i.e., lack prominent hairs, but there are many green algae whose flagella have prominent hairs called mastigonemes (Ringo, 1967). The Tribophyceae, Chrysophyceae, Phaeophyceae, Chloromonadophyceae, and Dinophyceae form two kinds of flagella, viz., (1) quite smooth, as in the Chlorophyceae and (2) hairy, i.e., provided with stiff (Chloromonadophyceae, Tribophyceae, Chrysophyceae (Fig. 7-1), and Phaeophyceae) or fine (Dinophyceae) hairs. The Euglenophycan flagella are provided with fine hairs,



whereas the Eustigmatophyceae, Cryptophyceae, and Bacillariophyceae produce only one kind of flagellum provided with stiff hairs.

### FLAGELLAR HAIRS

One or more types of flagellar hairs occur in most classes. The hairy covering seems to amplify the stroke of a flagellum because of the increased surface area. In many algal groups, the flagellar hairs are rather simple, i.e., they are thin, delicate, probably consisting of a single row of subunits. In the Cryptophyceae, Prasinophyceae, and certain heterokont algae, the hairs are tubular. These consist of two or more distinct regions, at least one of which is thick and tubular, while the distal elements often resemble simple hairs (Moestrup, 1982). Some heterokont algae have tripartite flagellar hairs which are attached to the flagellum in two opposite ranks. A tripartite hair consists of a basal part, a hollow shaft, and a distal part.

Many workers have examined the structure of the stiff hairs. These consist of three parts, viz., (1) a short basal portion, lying at an angle of about  $80^\circ$  to the long axis of the axoneme, (2) a tubular stiff shaft of about 15 nm diameter, often lying parallel to the axoneme, and (3) one or a few delicate terminal fibres, up to about  $0.7 \mu\text{m}$  long. Whereas the terminal fibres consist of one row of proteinaceous globular particles, the tubular shaft has 13 longitudinal rows of particles (Bradley, 1966; Bouck, 1971).

The hairs of euglenoid and dinoflagellate flagella are thin and non-tubular, form bundles, and are not differentiated into basal, central, and distal parts. The emergent flagellum of euglenoids has long hairs arranged in a single row and these hairs can be seen under a good light microscope. Besides these long hairs, euglenoid flagella have a dense felt of shorter hairs.

In dinoflagellates, the transverse flagellum carries unilateral hairs arranged

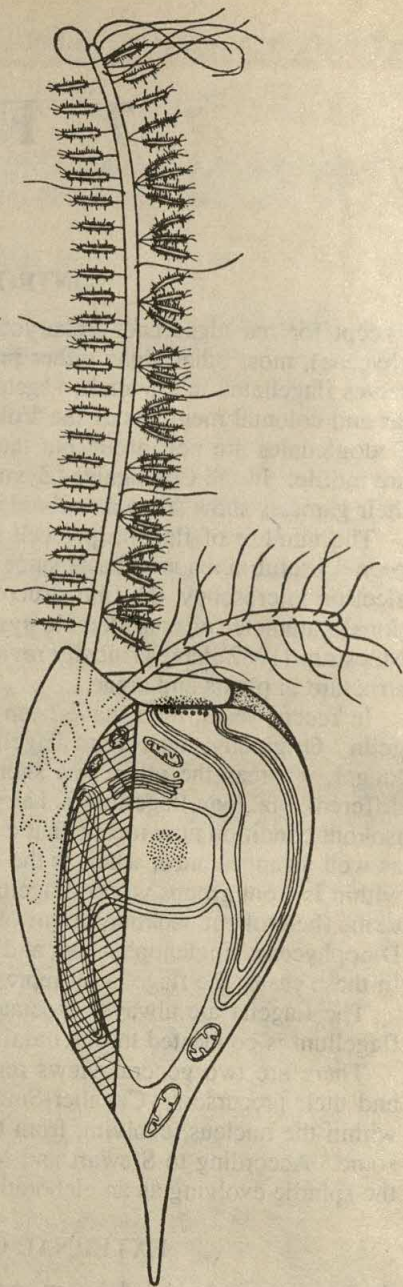


Fig. 7-1 Diagrammatic sketch to illustrate the fine structure of *Ochromonas* showing the arrangement of hairs on the two flagella. Note that the anterior flagellum has thick hairs covered with lateral filaments. (After Bouck, 1971.)



in bundles. The longitudinal flagellum also has hairs which are, however, much shorter than those on the transverse flagellum.

Thin and non-tubular hairs also occur in algae other than euglenoids and dinoflagellates but in these algae they do not form bundles.

The flagellar hairs of *Chlamydomonas reinhardtii* contain a glycoprotein which causes opposite mating types to agglutinate by their flagellar tips (Snell, 1976). This alga shows two types of adhesive phenomena associated with its flagellar surface. One occurs at initial stage of mating between gametes and is involved in sexual agglutination. A glycoprotein is involved in this agglutination. The second adhesive phenomenon is less specific as it is not confined to gametes but also occurs in vegetative cells. This event is associated with flagellar surface motility (Bloodgood, 1982) and involves gliding motility of the cells' interaction with a solid substrate via their flagellar surfaces.

At its anterior end, a *Chlamydomonas* cell has two cylindrical tunnels in its cell wall through which the flagella protrude. These tunnels are lined with 11 nm x 500 nm collars containing fibres disposed in a parallel array. The function of these fibrous collars appears to be to permit free movement of the flagella within the cell wall. Snell (1983) has shown that during the initial stages of gametic pairing, the intact collars slip off from the flagellar ends.

Members of the order Pavloales of the Haptophyceae characteristically have a dense covering of fine hairs around their flagella; such covering is lacking in other haptophytes.

The antherozoids of some brown algae, e.g., *Himantalia*, have their anterior flagellum provided with one or two spines. The spines are borne on the uppermost peripheral doublet of the axoneme and are surrounded by the flagellar membrane (Manton *et al.*, 1953).

The hairs of cryptophytes and the tripartite hairs of heterokontic algae are formed either within the perinuclear space of the nuclear envelope or in its extensions into the cytoplasm (Bouck, 1969; Loiseaux, 1973). The hairs are added to the base of the growing flagellum (the flagellum itself grows at the tip region).

In most algae, the hairs pass directly from the perinuclear space to the cell exterior in membrane-bound elements of the ER, but in *Ochromonas* (Fig. 7-2) and *Pilayella* they also move through the Golgi apparatus *en route*, and the Golgi apparatus seems to add some carbohydrate and glycoprotein component to these hairs (Loiseaux, 1973).

The flagella of some algae, e.g., *Ulvopsis*, *Phaeocystis*, *Hymenomonas*, and *Monomastix*, are covered with a surface coat or tomentum. A similar tomentum occurs underneath flagellar scales in spermatozooids of the Charales (Turner, 1968). The tomentum is especially conspicuous in *Chlamydomobotrys stellata* (Merrett, 1969).

## FLAGELLAR SCALES

These are mostly confined to the algae and are quite common in diverse algal classes. Most scales are unmineralized. Functionally, these scales may confer protection, have a role in cation metabolism or in sexual recognition (Melkonian *et al.*, 1981). In *Reckertia sagittifera*, only the short, anterior flagellum is covered with scales, whereas the posterior, trailing flagellum is naked.

In the Prasinophyceae, the flagella (4 or less) arise from an apical pit. They



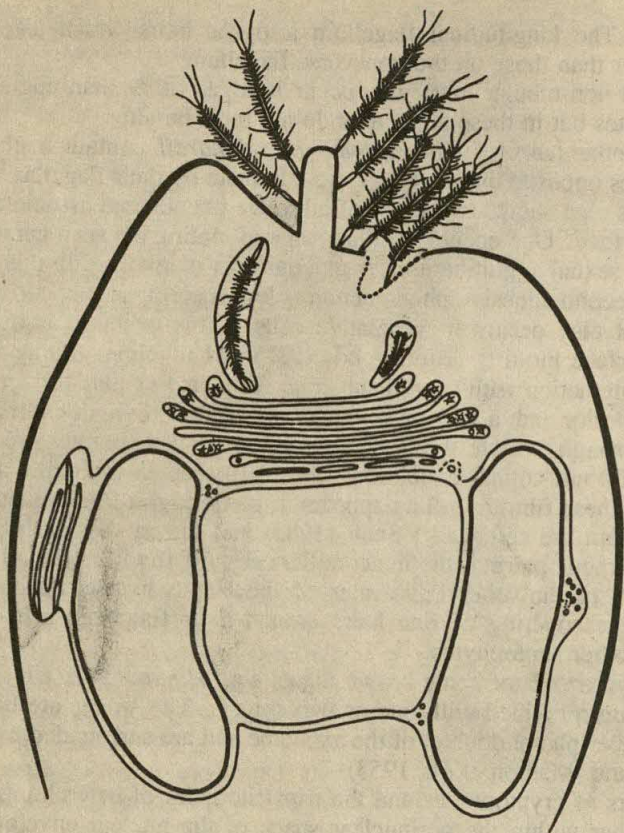


Fig. 7-2 Diagrammatic sketch of hair formation in *Ochromonas danica*. The hairs are produced in elements of endoplasmic reticulum and are later transported to the Golgi apparatus. Lateral elements seem to be added in the Golgi cisternae, and the fully formed hairs are transported to and released near the flagellar bases. (After Bouck, 1971.)

have a coating of scales as well as hairs. These flagella are thicker than those of the Chlorophyceae. In *Prasinocladus marinus*, the flagellar scales are arrayed in two layers, the flagellar bases are arranged in a row, the flagella are half the length of the body, and are usually directed forward in swimming. In this class, a special kind of fibrous band connects the flagellar bases to the chloroplast and nuclear envelope.

Flagella covered with tiny scales have also been found in the Chrysophyceae (Hibberd, 1973), Charophyceae (Moestrup, 1970; Turner, 1968), and some dinoflagellates (Clarke and Pennick, 1972). In the Chrysophyceae, the flagellar scales are not arranged in any regular patterns, and several different morphological types occur. They are quite small (less than 300 nm) and are probably made of acid polysaccharides. The long (30  $\mu\text{m}$ ), hairy flagellum of *Synura petersenii* can bear up to 1000 scales (Hibberd, 1973). Moestrup



(1982) showed that these scales are formed in the Golgi apparatus, though apparently not continuously. Some probable functions of the flagellar scales include defence, role in divalent cation metabolism, and sexual recognition.

### PARAFLAGELLAR ROD

In the Euglenophyceae, the free, emergent part of the flagellum often harbours a complex, elongated structure termed the paraflagellar rod, which runs parallel along the whole length of the flagellum (Fig. 7-3).

### INTERNAL STRUCTURE

Algal flagella show the same general internal structure as in those of several plants and animals. The main part of the flagellum is called the axoneme. In cross section (Figs. 7-4, 7-5), an axoneme is seen to have two central fibres or tubules and a ring of 9 peripheral doublet-tubules or tubule pairs. This arrangement is called the '9 + 2' pattern. The pseudocilia of *Tetraspora*, *Glaucocystis*, and *Gloeochaete* have a '9 + 0' structure. The shorter, non-emergent flagellum of *Euglena* also has a 9 + 0 arrangement. The spermatozooids of certain diatoms lack the central pair of tubules entirely, and those of *Golenkinia minutissima* have a single central

microtubule. Another common variation involves *additions* outside the 9 + 2 structure, such as the mastigonemes found in many algal flagella.

Each tubule pair has one tubule called A and one tubule called B. The A-tubules bear two small projections or side arms which point toward the B-tubule of an adjacent pair and are always directed or pointed clockwise. In some antherozoids (e.g., of *Lithodesmium*), the flagella lack central tubules, or they have only one such tubule, as in *Golenkinia* (Moestrup, 1972). Some paralyzed mutants of *Chlamydomonas* may lack an axoneme or may have only a short axoneme.

Certain radial spokes and appendages are associated with the central tubules. The radial spokes are rigid structures attached perpendicularly to the A-tubules. Sometime the heads on adjacent spokes contact each other, but the structures lack a general continuity along the flagellar length, and a better term

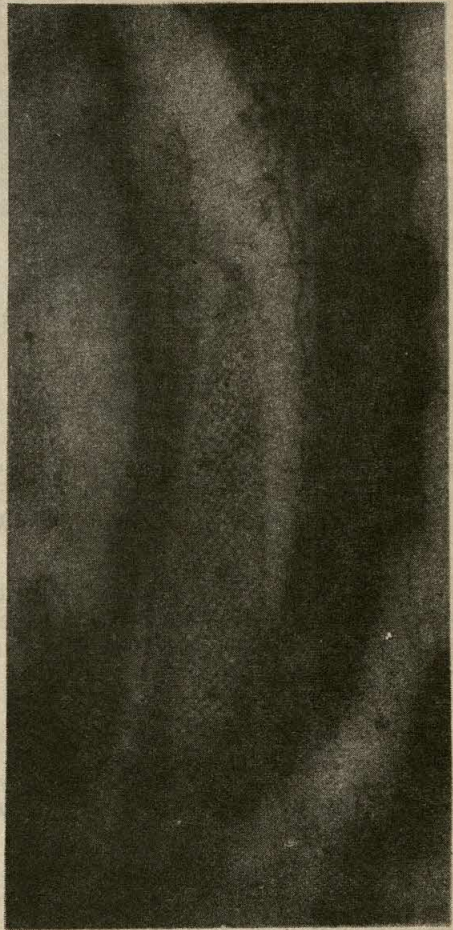


Fig. 7-3 *Trachelomonas*. Paraflagellar rod in the flagellum. (x42,000.) Courtesy K. Ueda.



for them is "spoke heads" (Gibbons, 1981). In flagella of *Chlamydomonas*, spokes occur in pairs with alternate spacings of about 32 nm between members of a pair and 64 nm between adjacent pairs.

Besides the radial spokes and the arms, the axoneme is held together by a set of circumferential linkages which join the centripetal side of each doublet tubule to that of the next.

Most algal flagella are cylindrical, around 0.25–0.35  $\mu\text{m}$  in diameter. The flagellar membrane usually fits smoothly around the axoneme, but in the flagella of *Haematococcus pluvialis*, *Ceratium hirundinella*, and certain volvocalean green algae, it fits loosely. In certain unicellular chrysophytes, the flagellar membrane extends to form a ribbon or wing supported along the edge by a paraxial rod (Swale, 1969).

In *Chlamydomonas reinhardtii*, the flagella are usually blunt. The central pair of microtubules continues to the flagellar tip but the peripheral pairs terminate one after the other near the tip, at first becoming singlets (Ringo, 1967). The flagella of *Friedmannia israelensis* zoospores have flat tips (Melkonian, 1983). In contrast, many members of the Haptophyceae, Chlorophyceae, and Loxophyceae have flagella with a pointed tip, called hairpoint. Fairly blunt flagellar tips are characteristic of most Cryptophyceae, Dinophyceae, Euglenophyceae, Prasinophyceae, Charophyceae, and Glaucophyceae (Moestrup, 1982). The hairy flagellum of most heterokont

pairs is very often blunt-tipped but in certain brown algae the tip ends in a hairpoint. The spermatozoid of *Desmarestia aculeata* has an exceptionally long hairpoint (Müller and Lütke, 1981). The hairy flagel-

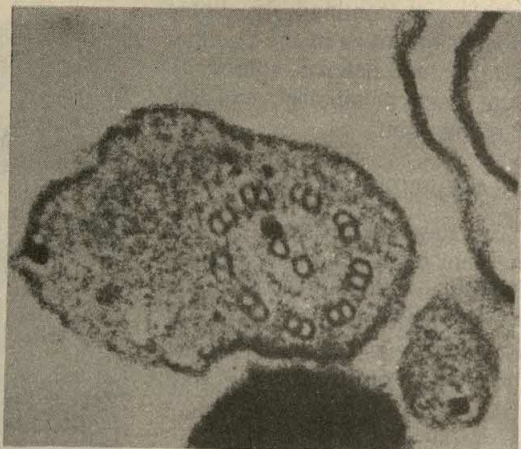


Fig. 7-4 Electron micrograph of cross section of a flagellum of *Euglena*, showing the 9 + 2 arrangement. (x100,000.) Courtesy K. Ueda.

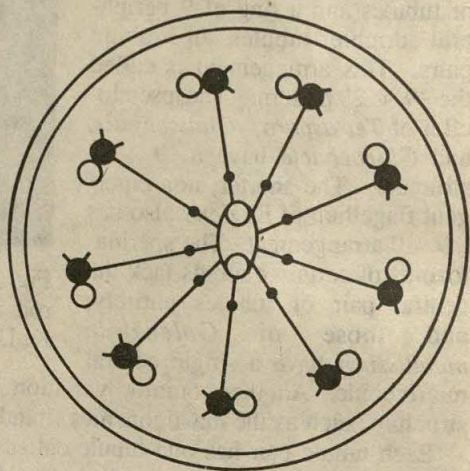


Fig. 7-5 Sketch of cross section through axoneme of a typical algal flagellum, showing the 9 + 2 arrangement and the two types of peripheral tubules.



lum of *Ectocarpus siliculosus* gametes has a 10–13  $\mu\text{m}$  long terminal hairpoint on the 10–15  $\mu\text{m}$  long flagellum. This hairpoint contains a continuation of the two central axoneme microtubules whose total length is therefore almost double of that of the peripheral pairs. In Phaeophycean gametes, the flagellar hairpoints or tips play an important role in mating. These flagellar tips are somewhat sticky and may have some proteins or glycoproteins involved in the recognition process.

### PARAXIAL INCLUSIONS

Sometime in addition to the 9 + 2 axoneme, flagella have other structures within the flagellar membrane. If these structures occur along the entire length of the flagella, they increase the flagellar diameter; if they stop short, they cause localized swellings. The additional structures tend to be located external to the axoneme in a paraxial position. In the Chrysophyceae, Xanthophyceae, Phaeophyceae, and Eustigmatophyceae, a swelling forms part of a photoreceptor apparatus. In the heterokont algae, excluding the Eustigmatophyceae, the flagellar swelling is located at the base of the smooth flagellum which often appears as a bulbous or stumpy structure. In the Eustigmatophyceae, the flagellar swelling is located near the base of the hairy flagellum and is T-shaped in *Vischeria helvetica* and *V. stellata*. The bar of the T is pressed against the eyespot while the trunk is connected to the flagellar axis.

In euglenoids, the flagellar swelling appears as a dense granule located near the transition region between the reservoir and the canal, some distance away from the flagellar bases.

Other kinds of flagellar swellings, which cannot readily be derived from eyespot-associated types, sometime occur in certain algae, e.g., *Urospora*, *Ochromonas*, and *Bicosoeca* (Moestrup, 1982). The swelling on the long flagellum of cryptophycean flagellates is a characteristic that sets apart the Cryptophyceae from the other algae. It is shaped like a platform and has a tuft of flagellar hairs directed anteriorly. The transverse flagellum of certain dinoflagellates contains a cross-banded paraxial rod, whereas their longitudinal flagellum contains a hollow rod. Euglenoid flagella also contain a long paraxial rod located close to the axoneme, where it seems to act as a straightening fibre.

The spermatozooids of *Dictyota* possess a longitudinal row of about twelve short spines on their single, hairy flagellum. Each spine is made of electron-opaque material, located between the flagellar membrane and the peripheral axoneme doublets, and the spines are attached to one of the doublets. Some Fucales, e.g., *Himanthalia*, *Xiphophora*, and *Hormosira*, also have spiny flagella.

### THE TRANSITION REGION

Although the flagellar axoneme and the basal body are remarkably constant in morphology in the algae, the transition region between them is highly variable. In the Cryptophyceae, this region has two close transverse partitions located some distance away from the level of the cell surface. The uppermost partition lies close to the origin of the central axoneme microtubules. The other often has a conspicuous central thickening.

In dinoflagellates, either two discs lie one above the other, or one large disc lies at the origin of the central axonemal microtubules, while one or two rings



are present further down below. Delicate, bent structures interconnect the two systems as, for instance, in *Gymnodinium micrum* and *Amphidinium* sp. (Dodge, 1973).

The third type of transition region is exemplified by *Ochromonas* and is common in several heterokont algae. It has a coiled fibre or transitional helix in addition to a single partition near the cell level. This helix in median longitudinal sections appears as a double row of punctae, representing the four to six turns of a helix. In *Mallomonas*, the transitional region of both flagella possesses a transitional helix distal to a transverse portion which lies at about the level of the cell surface. Some variability occurs in the number of gyres (usually 3 to 6). The longer of the two flagella is directed forward during swimming, lashing in an approximate sinus wave.

The *Prymnesium*-type of transition region is common in the Haptophyceae. There is no helix, and the transition region is quite different from that found in the heterokont algae. It contains two widely spaced, possibly interconnected, transverse partitions. A stellate structure is present in the area between the partitions and its arms connect with the A-tubules of the peripheral axonemal doublets.

The fifth type is illustrated by *Entosiphon* and is common in euglenoids. The whole basal body transition region seems empty, as also does the proximal part of the  $9 + 0$  axoneme.

*Entosiphon sulcatum* has the longest known basal body in the algae and has a very long transition region. The latter shows a single curved partition below the cell surface, and an extraordinary helical structure surrounds the proximal part of the central axonemal microtubules. This helical structure is a characteristic feature of most euglenoid flagella.

The green algal type of transition region occurs in all other chlorophyll b bearing algae. It has a typical stellate pattern divided into a longer distal and a shorter proximal part, separated by a transverse septum. The whole structure appears H-shaped in longitudinal section (Fig. 7-6).

The transition zone in the gametic flagella of *Acetabularia mediterranea* is fairly long, about 300 nm (Herth *et al.*, 1981). Ringo (1967) found that in *Chlamydomonas* the star-shaped structure lying around the basal part of the flagellar axoneme partly consists of a central cylinder to which certain triangular structures are attached. These triangular bodies establish contact with the A-tubules of the tubule pairs. A spiral structure occurs between the peripheral tubule pairs and the central part of tubules immediately above the basal disc in *Uroglena* (Caspar, 1972). This spiral body is not seen to establish contact with either the peripheral or the central tubules.

## BASAL BODIES

Most algae are remarkably uniform in respect of the structure of the basal part of their flagella. Basal bodies lack DNA but contain RNA and proteins (Friedlander and Salet, 1971). Most algal basal bodies have a fairly constant structure, i.e., a wall composed of nine tilted triplets and a proximal part containing the cartwheel pattern (Fig. 7-6). However, a cartwheel pattern is absent in *Volvox carteri* and zoospores of *Urospora* (Sluiman *et al.*, 1981) and also in most euglenoid flagellates. In the fully developed flagellum, the basal body probably functions in flagellar movement, though examples of motile flagella devoid of basal bodies are known, e.g., in *Chara*. Most algal basal bodies con-



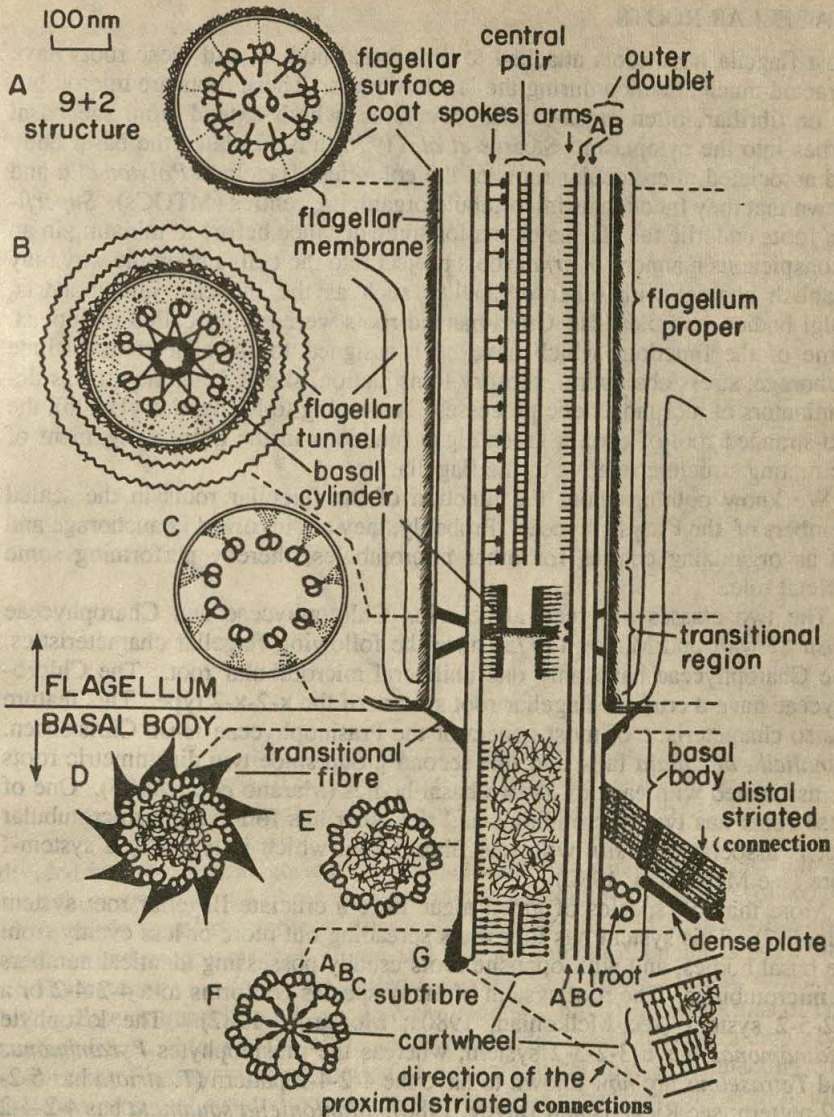


Fig. 7-6 *Chlamydomonas reinhardtii*. Diagrammatic reconstruction of flagellar shaft, transition region, and basal body, including flagellar roots, connecting fibres (after Cavalier-Smith, 1974). Not drawn to scale.

form to the 9 x 3 configuration.

The flagella of *Mallomonas* and *Synura* have their basal bodies arranged in parallel, whereas members of the Paraphysomonadaceae have the basal bodies disposed perpendicular to each other.

Certain connecting fibres interconnect basal bodies and probably occur in all groups of flagellate algae. These fibres may possibly function in some aspect of flagellar swimming (Hyams and Borisy, 1978).



## FLAGELLAR ROOTS

Most flagella have roots attached to their basal bodies, and these roots have attracted much attention during the last few years. These roots are microtubular or fibrillar, often cross-banded structures, which extend from the basal bodies into the cytoplasm. Stearns *et al.* (1976) have isolated the basal body and associated microtubular roots of the colourless flagellate *Polytomella* and shown that they function as microtubule organizing centres (MTOCs). *Superficial* roots underlie the plasmalemma for some distance before terminating in an inconspicuous manner. *Internal* roots project into the cell, and on the way may establish contact with other organelles such as the nucleus, mitochondria, Golgi bodies, and plastids. Cross-banded roots were earlier called rhizoplast. Some of the functions which have been assigned to flagellar roots include anchorage, stress absorption, sensory transduction, skeletal function, or as determinators of morphogenetic processes. According to Melkonian (1980b), the two-stranded root of certain green algae functions in the proper alignment of the mating structure relative to the flagella.

We know nothing about the function of the flagellar roots in the scaled members of the Chrysophyceae. Probably, they are involved in anchorage and act as organizing centres for other microtubules, thereby performing some skeletal role.

The two classes of green algae, viz., Chlorophyceae and Charophyceae *sensu* Stewart and Mattox (1975) have the following flagellar characteristics. The Charophyceae have only one unilateral microtubular root. The Chlorophyceae have a cruciate flagellar root system of the x-2-x-2 type. This feature is also characteristic of most genera of the Prasinophyceae *sensu* Christensen. *Dunaliella bioculata* falls into this second group since two disymmetric roots are associated with each of its two basal bodies (Marano *et al.*, 1985). One of these roots has two microtubules and the other has four. Each microtubular root is associated *in situ* with one fibrous root which is typically a system-I fibre (see Melkonian, 1980).

More than 50 species of green algae have a cruciate flagellar root system (Fig. 7-7). This system has four roots spreading out more or less evenly from the basal bodies, and with opposite roots usually possessing identical numbers of microtubules. The root system of many species conforms to a 4-2-4-2 or a 5-2-5-2 system (see Melkonian, 1980a; Moestrup, 1982). The loxophyte *Pedinomonas* has a 3-2-3-2 system, whereas the prasinophytes *Pyramimonas* and *Tetraselmis* are now known to have the 4-2-4-2 pattern (*T. striata* has 5-2-5-2 pattern; see Ricketts and Davey, 1980). *Mantoniella squamata* has 4-2-4-2 system.

Although cross-banded roots occur widely in the Loxophyceae, Prasinophyceae, and Chlorophyceae, they are rare or absent in the Glaucophyceae, Charophyceae, bryophytes or higher plants (Fig. 7-8).

According to Melkonian (1980a, b), the cruciate microtubular root system does not have much taxonomic or phylogenetic significance because it does not show any variations in different taxa of green algae. In contrast, the taxonomic value of the fibrous structures associated with the flagellar basal bodies is much greater because of the many variations seen in them. Melkonian recognizes two chief types of fibrous structures, viz., (1) those which connect different basal bodies and (2) those which originate at basal bodies and terminate somewhere else in the cell. The category (2) is further



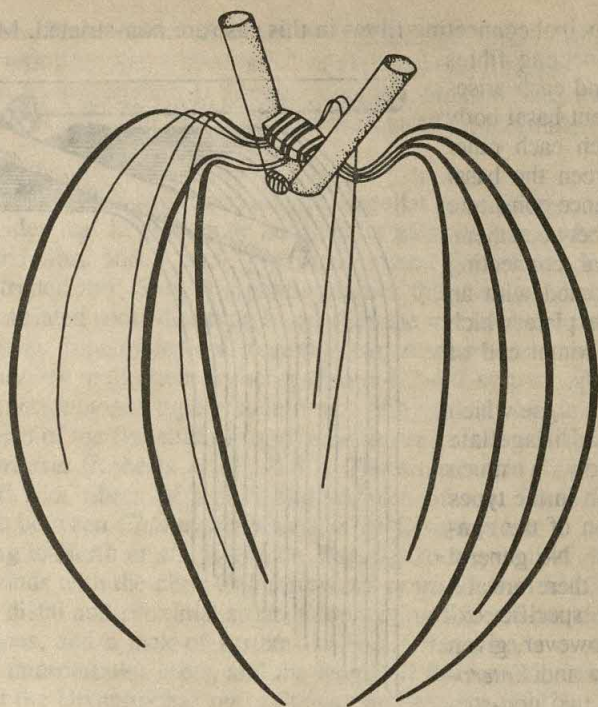


Fig. 7-7 The microtubular root system typical of most green algae. It comprises four roots. The 4-2-4-2 system of *C. reinhardtii* is shown here. (After Ringo, 1967.)

divided into two subcategories: (a) striated fibres associated with microtubular root; these are system-I fibres with a striation pattern 25–35 nm and (b) striated fibres made up of a bundle of filaments; these are system-II fibres having a striation pattern greater than 80 nm and whose filament diameter is 5–10 nm.

The category (1) fibres are called the connecting fibres. Algae producing biflagellate or quadriflagellate swimmers generally have different kinds of connecting fibres. Many biflagellate swimmers produce the Chlamydomonad-type connecting fibre systems consisting of a single distal striated connecting fibre and two proximal striated connecting fibres. The movement of the individual flagella is thought to be coordinated by the distal fibre but the function of the proximal fibres is not known. Examples occur in species of *Chlamydomonas*, *Polytoma*, *Chlorogonium*, *Dunaliella*, *Tetraspora*, *Pediastrum*, and *Hydrodictyon*.

Striated fibrous structures originate at flagellar basal bodies, terminating somewhere in the cell. In *Chlamydomonas reinhardtii*, there is a single continuous fibre overlying 2-stranded roots that are associated with microtubules (Goodenough and Weiss, 1978). The fibres are closely associated with the cruciate microtubular roots and run parallel to the root microtubules. The fibre which overlies the two-stranded root in gametes is in direct contact with the mating type structure and may function in signal transmission during mating.

Another type of connecting fibre found in biflagellate swimmers is characteristic of *Bryopsis* and also occurs in *Microthamnion*, *Ulva*, and *Enteromor-*



*pha*. The principal connecting fibres in this case are non-striated. Mostly, two principal connecting fibres are present and each arises from a different basal body but they touch each other midway between the basal bodies and hence constitute a connection between them. Each principal connecting fibre is associated with an electron dense plate which closes the proximal end of each basal body.

Those green algae which produce quadriflagellate swimmers show a much wider variation in the types and disposition of the connecting fibres. No general account can therefore be given. Two specific examples are, however, given here. In *Ulva* and *Enteromorpha*, only two non-striated connecting fibres connect the adjacent basal bodies of different pairs of basal bodies (Melkonian, 1980a, b). In *Stigeoclonium* and *Fritschiella*, adjacent basal bodies seem to be linked by non-striated connecting fibres and, besides these, at least two proximal striated connecting fibres link adjacent bodies in some species. In *Schizomeris*, adjacent basal bodies are connected by a cross-banded structure resembling the distal fibre in *Chlamydomonas* (Birkbeck *et al.*, 1974), and at their proximal ends, adjacent basal bodies are connected by proximal fibres. According to Birkbeck *et al.*, the flagellar apparatus of *Schizomeris* zoospores greatly resembles that of the Oedogoniales which also have a prominent ring of cross-striated fibrils connecting the basal bodies (Hoffman and Manton, 1963).

**Category (2) fibrous roots** Moestrup (1978) has listed the fibrous roots found in green algae. Fibrous roots do not occur in the Charophyceae and are also absent in the Bryophyta and other higher plants.

**System-I fibres** These are associated with cruciate microtubular roots and run parallel to the root microtubules. Examples of green algae having system-I fibrous roots (including both biflagellate and quadriflagellate swimmers) are *Chlamydomonas*, *Dunaliella*, *Cylindrocapsa*, *Enteromorpha linza*, and *Ulva lactuca*.

**System-II fibres** These are composed of a bundle of fine filaments interrupted by cross striations. They are generally not closely associated with micro-

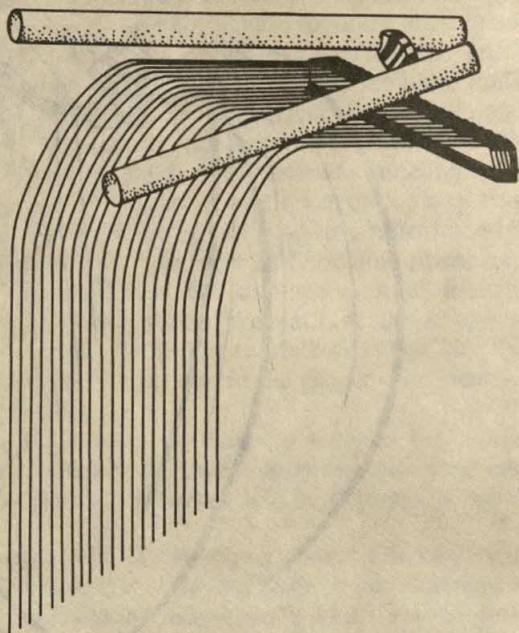


Fig. 7-8 The microtubular root system of charophytes, bryophytes, and higher plants. It has a single large root, which, near the flagellar bases, is often associated with a lamellate structure. (After Moestrup, 1982.)



tubular roots. These fibres often run towards the cell nucleus and in this case very often represent the "rhizoplasts" of classical phycologists. Examples of green algae having system-II fibrous roots are *Monostroma*, *Prasiola*, *Urospora penicilliformis*, *Carteria*, *Pyramimonas*, and *Prasinocladus* (Norris *et al.*, 1980).

Herth *et al.* (1981) have found that the flagellar root system of *Acetabularia* gametes is identical in both male and female gametes. In both, a prominent distal striated fibre and a small proximal striated fibre connect the flagellar bases. A striated root fibre of type#I underlies the microtubular root type#II, and a short striated root fibre type I underlies the microtubular root type#I.

The flagellar root system of *Acetabularia mediterranea* is a modified *Chlamydomonas*-type system in being also a 4-2-4-2 system. It differs from the true *Chlamydomonas*-type system in not being strictly cruciate. As regards the attachment of the flagellar roots, *A. mediterranea* is more like *Ulva*, *Bryopsis*, and *Derbesia* (Roberts *et al.*, 1980). The situation in *Acetabularia* with four striated root fibres of type#I and absence of root fibres of type II is intermediate between *Chlamydomonas* and the Ulvophyceae.

According to Herth *et al.* (1981), the flagellar root system of *A. mediterranea* corresponds with the class Chlorophyceae *sensu* Stewart and Mattox in the presence of distal and proximal striated connecting fibres, absence of microtubule septations, and a lack of system-II fibres. Basal body overlap, relative insertion of microtubular roots, and the length of the transition zone are more like those in the Ulvophyceae *sensu* Stewart and Mattox (1978).

Roberts *et al.* (1980) have suggested that the siphonous green algae should be placed in the Ulvophyceae because of similarities in the components of the flagellar apparatus between the motile cells of ulvacean and siphonous algae. Both male and female gametes of *Derbesia tenuissima* have flagella with a cruciate microtubular root system, a non-striated capping plate which connects basal bodies, two electron dense terminal caps which partially cover the proximal end of the basal bodies, and two small system-II fibrous roots (Roberts *et al.*, 1981a, b). These components are remarkably similar to those in ulvacean swimmers. Because of these similarities, Roberts *et al.* suggest the inclusion of the order Caulerpales in the Ulvophyceae rather than in the Charophyceae or Chlorophyceae.

The flagella of the gametes of *Trentepohlia*, *Cephaleuros*, and *Phycopeltis* have the following features that are unique for green algae: (1) They bear lateral keels formed by extension of the plasmalemma around certain microtubule-like fibres; one of which occurs in each keel. (2) Their insertion is peculiar, being apical, but is neither strictly bilaterally symmetrical nor asymmetrical; Chapman (1980) has designated this kind of flagellar insertion as "reversed bilateral symmetry". (3) The morphology, number, and arrangement of multilayered structures are collectively rather distinctive.

In *Cephaleuros virescens*, the flagella lack scales, are smooth, isokont, keeled, apically inserted, having parallel basal bodies which overlap laterally (Chapman, 1980). Each basal body is associated with a separate multilayered structure and a component microtubular "spline" (or spline-mitochondrion). The spline extends posteriorly beneath the plasma membrane. In this species, the four flagella on the zoospores also are smooth, isokont, bilaterally keeled, scale-less, with typical "9 + 2" axoneme. They are apically inserted, with the parallel basal bodies overlapping laterally at two levels (Chapman, 1981).



Diagonally opposing upper and lower basal bodies harbour flagella which protrude from the same side of the apical papilla. Each of the four basal bodies is associated with a microtubular spline, and the latter extends beneath the plasma membrane right through to the posterior end of the zoospore. A distinct multilayered structure is associated with each of the lower basal bodies. Like gametic flagella, the zoosporic flagellar insertion is reversed bilaterally symmetrical. [Floyd *et al.* (1980) term the "reversed bilateral symmetry" as "180° rotational symmetry".]

Another apparently unique feature of the motile cells of *Cephaleuros* and other members of the Trentepohliaceae is the presence of a densely staining collar associated with the plasmalemma at the flagellar insertion (Chapman, 1983). However, although *Cephaleuros*, *Trentepohlia*, and *Phycopeltis* possess several unique features, if these members were to be placed in one of the classes of the green algae proposed by Stewart and Mattox (1978), they would better be placed in the Ulvophyceae (Chapman, 1983) than in either the Chlorophyceae or Charophyceae.

In the Cryptophyceae, the flagellar roots contain a characteristic *rhizostyle* which is a single internal flagellar root which passes from one of the flagellar bases deeply into the cell, along the gullet, behind the trichocyst layer, terminating in the posterior half of the cell. *En route*, it contacts with the nucleus by running through a groove in the nuclear surface (Fig. 7-9) (see Roberts *et al.*, 1981a, b).

The root apparatus in the Dinophyceae is generally complex in respect of the number and morphology of the ancillary structures associated with the one (e.g., *Ceratium hirundinella*) or two (*Glenodinium foliaceum*) microtubular roots. In *Amphidinium* (Figs. 7-10, 7-11), a fibrous multilayered structure joins the single microtubular root. In *A. cryophilum*, a striated collar (Fig. 7-10) is associated with each flagellar canal (Wilcox *et al.*, 1982). In *Oxyrrhis*, two dense swellings are associated with one large microtubular root and, in addition, in an adjacent root a semi-circular row of microtubules partly covers a dense core.

Several microtubular roots and a single large cross-banded root are typically found in the chrysophycean flagellates. The cross-banded root originates near the flagellar bases and passes deeply into the cell where it becomes associated with the nucleus, the Golgi apparatus, and sometime also with the chloroplast. In most chrysophytes, the microtubular roots are fairly inconspicuous, having only a few microtubules.

In the fusiform spermatozoids of the diatom *Biddulphia levis*, a system of microtubules (probably flagellar roots) radiates into the cell from the single basal body present, and forms a cap on the anterior part of the nucleus. Cross-banded roots are lacking.

The typical root system in the Xanthophyceae consists of three parts, viz., (1) a descending root, (2) a cross-banded fibrillar structure, and (3) a number of microtubular roots. The descending root is unbranched and consists of a series of rectangular blocks (about 20 nm x 40 nm) spaced about 20 nm apart. The cross-banded fibrillar structure seems to arise together with, but at right angles to, the descending root, and terminates at the plasmalemma. It is made up of slightly curved bands whose widths taper off towards the point of contact with the basal bodies.

Neither descending root nor cross-banded fibrillar structure is present in *Vaucheria*, but its proboscis is supported by a single broad microtubular root



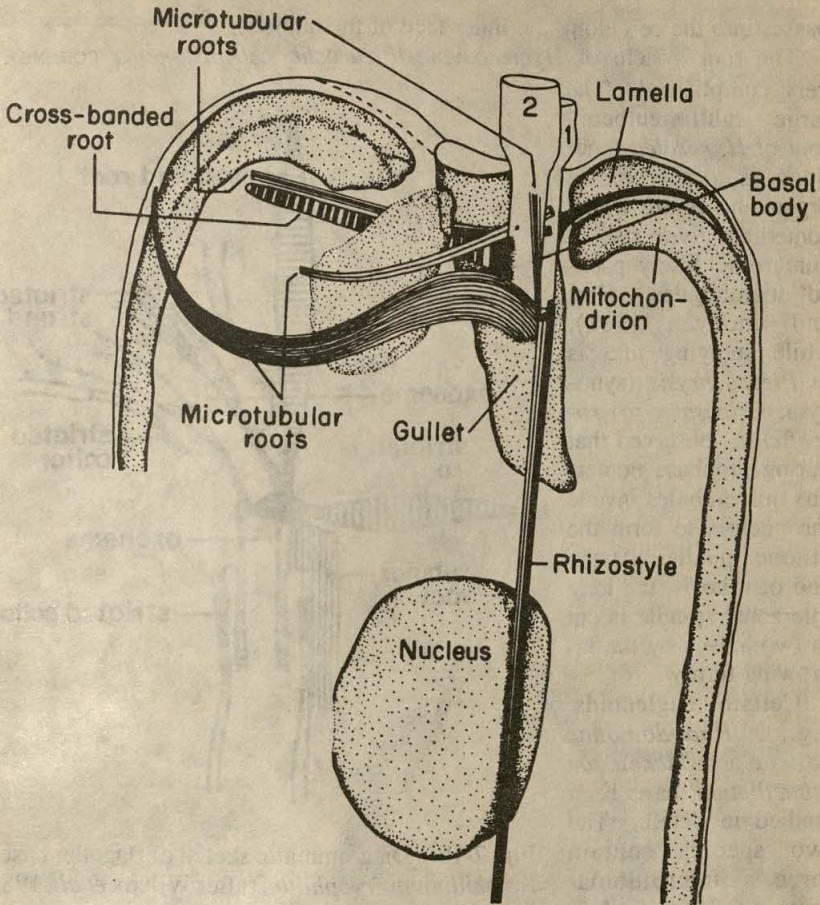


Fig. 7-9 *Chilomonas paramecium*. Diagrammatic reconstruction of the complete flagellar root system, including rhizostyle, a cross-banded root, and several microtubular roots. (After Moestrup, 1982.)

which has 8 or 9 microtubules arranged in a row. A second group of 1-4 microtubules possibly represents another flagellar root. So far as the structure of the flagellar root is concerned, *Vaucheria* resembles brown algae rather than the Xanthophyceae.

In some primitive brown algae, the flagellar root is fairly narrow, usually comprising only up to 8 microtubules, and at the anterior end of the cell turns back to run along the same side of the cell, along the ascending part of the root, before bypassing the basal bodies and then ending near the eyespot. In spermatozooids of *Fucus*, a kind of 'proboscis root' supports the flat and elastic proboscis; this root (Fig. 7-12) is quite broad, having 12-15 microtubules which travel from the base of the front flagellum along the plasmalemma to the anterior end of the cell. Here they bend back, run along the plasma membrane on the opposite side. The proboscis serves a sort of skeletal function.

In the Prymnesiophyceae (Haptophyceae), the Pavlovales have a fibrous, non-striated root which extends from the base of the anterior flagellum and



passes into the cell along the inner face of the nucleus.

The root system of *Hymenomonas/Pleurochrysis/Apistonema* complex is very complicated. The large multimembered root of *H. rosalea* functions as a microtubule organizing centre which sometime gives rise to numerous closely packed microtubules. Hori and Inouye (1981), while studying mitosis in *Pleurochrysis* (synonym: *Cricosphaera*) *roscoffensis*, observed that during prophase numerous microtubules invade the nucleus to form the mitotic spindle. At the end of mitosis, the long interzonal spindle is cut in two halves by the ingrowing furrow.

Certain euglenoids, e.g., *Rhabdomonas costata* and *Menoidium bibacillatum*, have been studied in detail. The two species contain three microtubular roots, a 6-8-2 and a 5-8-

5 system, respectively, with all three roots extending from the basal bodies along the reservoir. Contrary to a fairly popular but erroneous belief, the root tubules are not continuous with the microtubules of the canal.

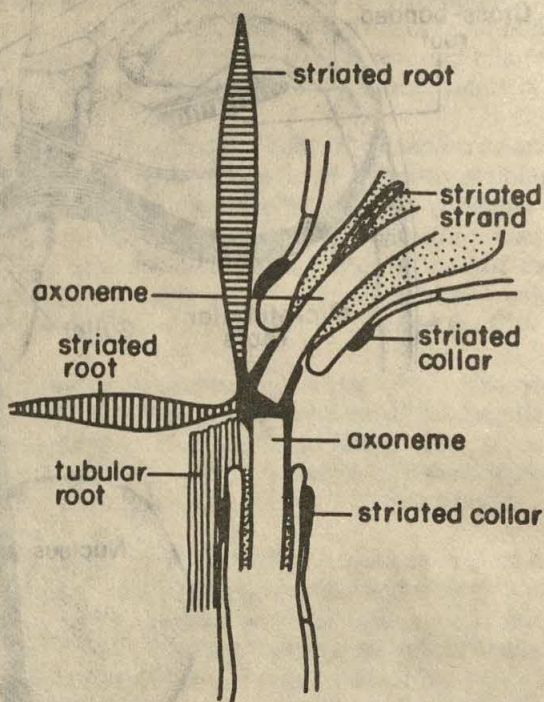


Fig. 7-10 Diagrammatic sketch of flagellar base of *Amphidinium cryophilum* (after Wilcox *et al.*, 1982).

## THE HAPTONEMA

The Haptophyceae have a third flagellum-like structure called haptonema. The term haptonema is used here because the internal structure of this organelle differs from the flagellar 9 + 2 arrangement; the haptonema in cross section is seen to have three concentric membranes which enclose 6 or 7 microtubules. Unlike flagella, the haptonema does not have any roots. *Prymnesium parvum* has a very short stump-like haptonema, whereas *Chrysochromulina parva* has a haptonema longer than 60  $\mu\text{m}$  (Parke *et al.*, 1962).

## COMPOSITION

Axonemes contain an ATPase protein responsible for mechano-chemical energy transduction, and a structural protein of the tubules. At least a part of the ATPase is located in the arms. Since the axonemal ATPase is physico-chemically different from muscle ATPase or myosin, a new name (dynein) has been given to it. Dynein occurs as two or three isozymes. Dynein specifically hydro-



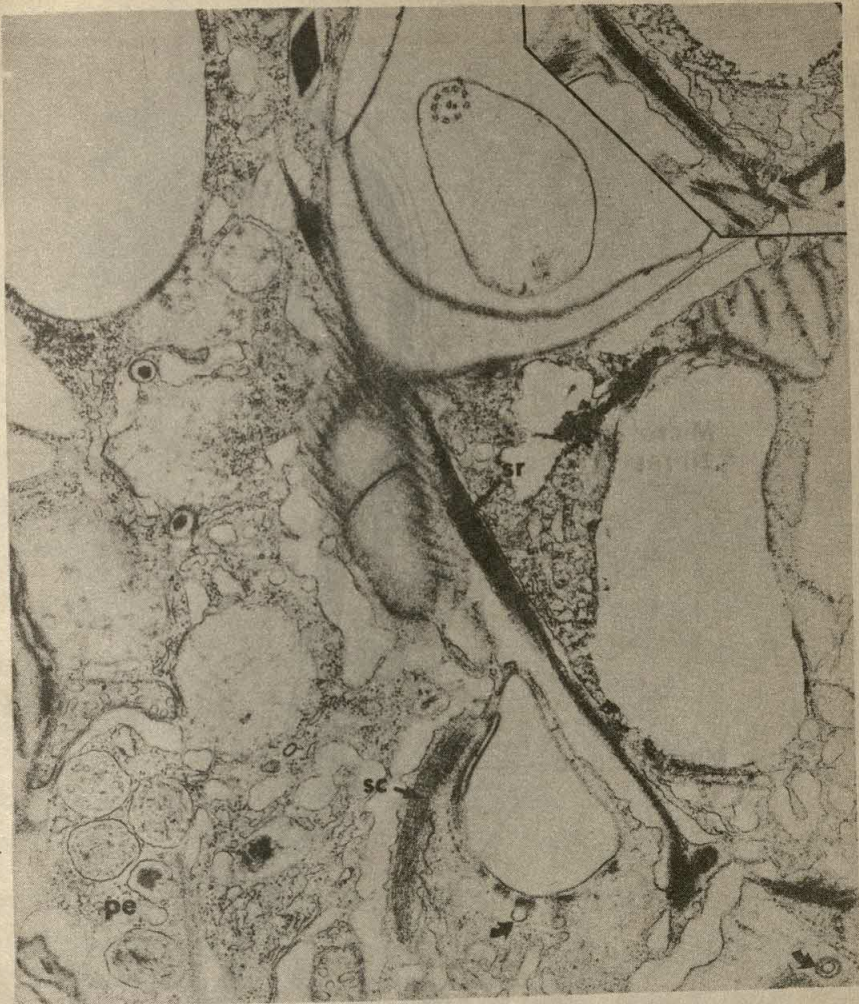


Fig. 7-11 *Amphidinium cryophilum*. Non-adjacent serial sections of the transverse flagellar base region. Curved arrows show collared pits, in cross and longitudinal section. Straight arrows show tubular root. Portions of both basal bodies are visible at the top right-hand corner. The typical 9 + 2 arrangement in cross section is seen at top middle. pe, peduncle; sc, striated collar; sr, striated root. (x21,450.) Courtesy L.W. Wilcox.

lyzes ATP in preference to other nucleotides, requires magnesium or calcium ions for its activity, and is inhibited by EDTA. These characteristics correlate closely with those required for axonemal reactivation.

The dynein extracted from *Chlamydomonas* flagella showed two forms sedimenting at 13S and 18S. An electrophoretic study of *Chlamydomonas* axonemes revealed 10 bands with molecular weights ranging from 300,000 to 330,000 daltons (Piperno and Luck, 1979a). The axonemes of this alga not only contain dynein and tubulin (the two major proteins) but also as many as 180 minor polypeptides, occurring in traces (Piperno and Luck, 1979b; Piperno



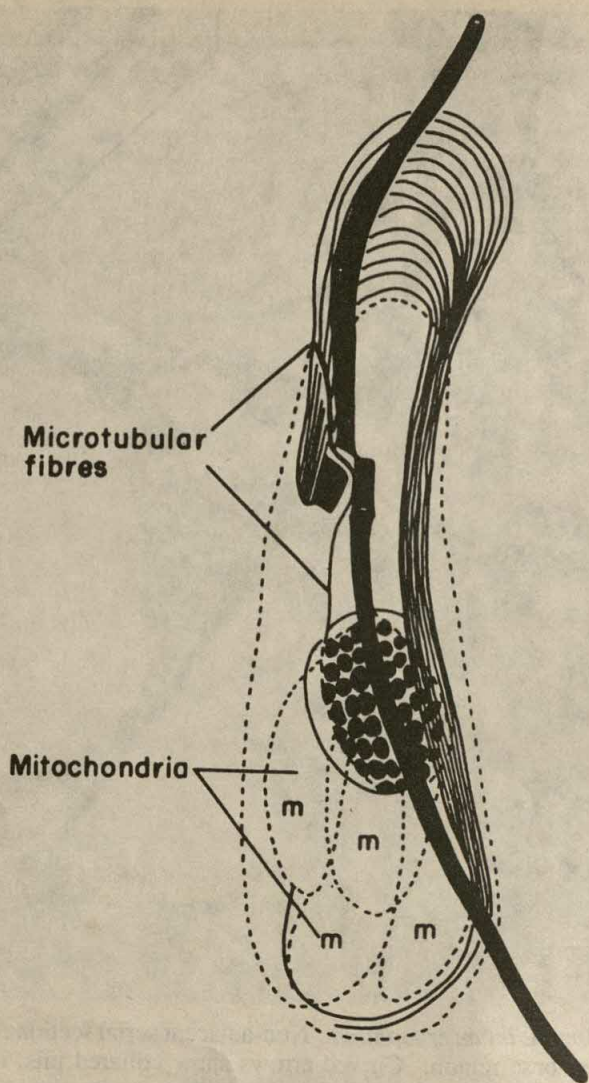


Fig. 7-12 Antherozoid of *Fucus serratus*. It has a broad microtubular root which supports the 'proboscis'. (After Manton and Clarke, 1956.)

*et al.*, 1977).

Some recent researches suggest that a dynein-like ATPase may be implicated in movements associated with cytoplasmic microtubules which have also been shown to bind to dynein (Haimo and Rosenbaum, 1981).

#### FUNCTIONS OF THE COMPONENTS OF THE FLAGELLAR APPARATUS

According to Salisbury and Floyd (1978), the striated rhizoplast of *Tetraselmis* (= *Platymonas*) initiates, coordinates, or adds directional control to the flagel-



lar stroke. If some swimmers lack rhizoplasts, then these functions may be carried out by the striated connective structure between basal bodies. These views are, however, not supported by the work of Bessen *et al.* (1980) who found that flagella can beat without being connected to the cell and therefore do not need any stimulus from these striated structures. According to Bessen *et al.*, striated structures may help the anchorage of the flagellar apparatus where an ability to contract may be necessary. Roberts *et al.* (1981a, b) feel that the system-II roots, rhizoplasts, and striated connective structure between basal bodies probably act in absorbing the shock produced by the beating of flagella, thereby preventing disruption of the cell in the immediate vicinity of the basal bodies.

### MECHANISM OF FLAGELLAR MOVEMENT

Afzelius (1959) suggested a sliding-tubule model in which flagellar bending was based upon the relative sliding movement between adjacent doublets as a result of activity of the arms on the doublets. This model received much support from the work of Satir (1968). The evidence for the occurrence of active sliding between flagellar tubules is now considered conclusive (Gibbons, 1981). Certain mutants of *Chlamydomonas* which lack either the radial spokes or the central tubules are paralyzed; this points to the major role of these structures in coordinating the sliding among the outer doublets and converting it into bending.

Unlike the breaststroke-like motion of the unicellular *Chlamydomonas*, such colonial green algae as *Astrephomene* show a different mode of flagellar action that has been described by Hoops and Floyd (1983). The effective stroke lasts about as long as, or just slightly longer than, the recovery stroke. Often a flagellum stops near the start of the effective stroke, for a considerable period, before resuming again. The direction of the effective stroke is towards the posterior of the spherical colony, varying little for a given flagellum. Both the flagella of any given cell tend to beat in parallel planes in the same direction. During development, an electron dense strut forms near a two-membered flagellar rootlet and grows past basal bodies. The plane of the effective stroke is more or less at right angles to this strut, but never reaches that position (Hoops and Floyd, 1983). In *Astrephomene gubernaculifera*, the flagella of any given cell are not necessarily synchronized. Often, one flagellum is in the middle of the effective phase while the second happens to be in the middle of the recovery phase. Sometime, both flagella stop beating for a while; later, one starts the effective stroke and is immediately followed by the second.

Recent researches have also revealed that calcium ion exerts a strong regulatory effect on the beating of flagella. Increased calcium causes decreased asymmetry in *Chlamydomonas* flagella (Gibbons, 1981). Calcium level also affects the length of the cross striated fibre (which anchors the basal bodies of each of the two pairs of flagella to the plasmalemma) in *Platymonas*; when this alga is fixed in the absence of calcium, this fibre is about 2.2  $\mu\text{m}$  long, but when fixation is done in the presence of 1 mM of  $\text{CaCl}_2$ , the fibre contracts to less than 1  $\mu\text{m}$  (Salisbury and Floyd, 1978). The shrinkage of this fibre may cause changes in angular orientation of the basal region of the flagella during swimming.

According to Gibbons (1981), the normal beating of flagella results from active sliding movements between adjacent doublets of the axoneme. This



sliding is powered by an ATP-driven mechano-chemical cycle in which dynein arms on one doublet interact with successive binding sites along the B-tubule of the neighbouring doublet, and are coordinated and resisted by the radial spokes that convert the sliding into bending.

Since the spatial periodicities of associated proteins along the axoneme are very precise, a temporal control of their biochemical activities may be the answer (Bornens, 1980). According to Bornens, the centriole controls the movement of the cell. This view assumes that: (1) microtubules are conducting structures capable of transmitting signals and (2) centriolar cylinders rotate around their axes. Bornens (1980) has proposed a hypothesis to explain the movement of flagella. The essential postulates of this hypothesis are: (1) A-subfibres are conducting structures that can transmit signals, (2) B-subfibres tangentially deflect the signals in (1), (3) the central doublet of the 9 + 2 rotates and permits a temporal control of the interaction between the central sheath and the radial spokes, and (4) the kinetosome rotates around its axis but the axoneme does not. This generates periodic signals at the bases of the 9 A-subfibres. Bending waves may be produced by the axoneme alone, with the kinetosome acting as a regulator.

Many unicellular flagellates, such as *Chlamydomonas*, characteristically use a breaststroke-like motion. In *C. reinhardtii*, the flagellar beat cycle consists of two phases: (1) an *effective* phase, involving the formation of a principal bend near the base of the flagellum while the straight distal part of the flagellum sweeps through the ambient water, and (2) a *recovery* phase, during which the basal bend propagates distally, making the flagellum virtually straight and pointed towards the anterior. According to Brokaw *et al.* (1982), the flagellum also generates reverse bends, which propagate along itself in alternation with the principal bends. The principal and reverse bends are regions in which the sliding between flagellar microtubules occurs in different directions. The principal bends differ from the reverse bends in having a much greater sliding velocity (Brokaw *et al.*, 1982). It is the asymmetry between the principal and the reverse bends that is partly responsible for the flagellar movement.

During flagellar beating, the cells rotate. Studies on a uniflagellate mutant of *C. reinhardtii* have shown that the effective phase of the flagellar bending cycle, during which the cell rotates, occupies about two-third of the bending cycle (Brokaw and Luck, 1983). It commences some 0.1 cycle after start of the sliding in the new reverse bend that causes the forward rotation of the cell and the generation of a new principal bend at the flagellar base. In this new principal bend, sliding begins about 0.1 cycle before the end of the effective phase and the start of the recovery phase. The recovery phase involves a backward rotation of the cell which results in about 25–30% loss of the forward rotation that occurred in the effective phase (Brokaw and Luck, 1983).

In contrast to the forward beating mode, the reverse beating mode involves the propagation of fairly symmetrical, undulatory bending waves along the flagellum from base to apex, with bends that appear less marked than the principal bends observed in the forward mode (Goldstein, 1982; Brokaw and Luck, 1983). Flagellar reversal is important in the context of the cell's response to environmental stimuli such as strong light. During reverse beating, the flagella are directed anteriorly, with the axis of symmetry of the bending pattern being intimately aligned along the long axis of the cell.



## PHYLOGENETIC IMPLICATIONS

Flagellar structure furnishes important clues to the phylogeny of major algal groups. The dinoflagellates are quite conspicuous, having different types of flagellar hairs, arranged in different ways on the longitudinal and transverse flagella; they have a unique type of flagellar transition region, and contain paraxial flagellar rods. This distinctive assemblage of flagellar characters rules out any possible relationship between dinoflagellates and other chromophyte algal groups. The cryptophytes also occupy an isolated position, and the idea to group them along with dinoflagellates deserves to be abandoned: the flagellar roots, transition region, and the location of flagellar swelling in cryptophytes are quite unlike those in dinoflagellates. The combination of tubular hairs in one row on one flagellum and in two rows on the other flagellum is a trait that clearly sets the cryptophytes apart from all other algal groups. Thus, these algae deserve a separate phylum, the Cryptophyta.

Algae with heterokont flagella seem to constitute a fairly close and natural coherent group which may be called the Heterokontophyta (Moestrup, 1982); the Eustigmatophyceae could also perhaps be included in this phylum because of close similarities in flagellar structure.

The Haptophyceae superficially resemble the Chrysophyceae in some respects but differ in respect of cell structure as well as flagellar transition region.

Among the algae with chlorophyll *b*, the euglenoids conspicuously stand apart from the others in cytology as well as distinctive flagellar characters, such as the complex hairy covering of the flagellum, the unique type of paraxial rods for chlorophyll *b* containing algae, and the very simple flagellar root system.

Emphasizing the value of flagellar characteristics, Moestrup (1982) has suggested that the potentially-flagellate algae may be classified into seven phyla, viz., Cryptophyta, Dinophyta, Heterokontophyta, Haptophyta, Glaucophyta, Euglenophyta, and Chlorophyta. The last three of these may even be grouped together in a single super-phylum. According to Moestrup, the flagellar characters do not warrant the splitting of the Chlorophyta into Chlorophyceae, Charophyceae, etc.

During the course of a few years, phycologists have recognized the great significance of the absolute orientation of the flagellar apparatus of motile green algae showing 180° rotational symmetry. The orientation patterns are based on the displacement of certain flagellar apparatus components relative to a strictly cruciate arrangement, when the apparatus is seen from the anterior end of the cell. The counterclockwise-clockwise absolute orientation proposal for defining the arrangement (O'Kelly and Floyd, 1983) describes the apparent rotation of basal bodies and rootlets in cells away from the hypothetical cruciate arrangement. The Ulvaphyceae typically show the counterclockwise arrangement, whereas many Chlorophyceae show the clockwise configuration. O'Kelly and Floyd (1984) attach much phylogenetic significance to the counterclockwise-clockwise configuration. The counterclockwise absolute configuration is the only configuration met with in the Charophyceae, Prasino-phyceae, and Ulvaphyceae, but also occurs in some members of the Chlorophyceae. The scale-bearing zoospores of *Chaetopeltis* (Chlorophyceae) show a configuration that is virtually strictly cruciate and, hence, probably ancestral and primitive. Clockwise absolute orientation seems to be the rule in many



Chlorophyceae, but some exceptions which show counterclockwise configuration (e.g., *Hafniomonas*, *Tetraselmis*, *Friedmannia*) are known.

Ultrastructural investigations of several algae during the last few decades have not only revealed the heterogeneity of such well known classes as Chlorophyceae, but also of several orders, families and even genera. The Chlorophyceae *sensu* Fritsch is now generally split up into the rather primitive Prasinophyceae (which itself may be an unnatural assemblage), and the advanced classes Chlorophyceae, Ulvophyceae, and Charophyceae. The higher plants are thought to have evolved from ancestral progenitors resembling the Charophyceae. *Ulothrix* exemplifies an unnatural genus. In lacking phycoplast as well as the unilateral multilayered structure-associated flagellar roots, *U. zonata* and *U. tenuissima* can neither be placed in the Chlorophyceae nor in the Charophyceae, but are best placed in the Ulvophyceae (Sluiman *et al.*, 1983), as their general mode of cell division resembles that in such other members of the Ulvophyceae as *Ulva*, *Enteromorpha*, and *Monostroma*. In contrast, *U. fimbriata* and *U. belkai* are patently Chlorophycean in possessing a typical phycoplast. The flagellar apparatus of *U. belkai* is unlike that of *U. zonata* and several Ulvophyceae but is more akin to that of such Chlorophyceae as *Stigeoclonium*, *Schizomeris*, and *Draparnaldia*. According to Sluiman *et al.* (1983), *U. belkai* and *U. fimbriata* should be removed from *Ulothrix* and transferred to *Uronema* and *Stigeoclonium*, respectively.

The possible nature of the ancestral green alga has been of interest to many phycologists, especially after the revelation of fine structural details in several green algae. According to O'Kelly and Floyd (1984), the ancestral green algal flagellate cell showed counterclockwise absolute orientation of the flagellar apparatus and had diamond-shaped scales. However, whether the ancestral flagellate had two flagella or four is not certain; Melkonian (1982) favours the biflagellate condition whereas Stewart and Mattox (1980) support the quadri-flagellate condition, with O'Kelly and Floyd (1984) also inclined toward the quadri-flagellate view.

O'Kelly and Floyd (1984) believe that the Chlorophyceae and Ulvophyceae are closely related and may have descended from a common ancestor that possessed scales, a flagellar pit, and a quadri-flagellate flagellar apparatus having 180° rotational symmetry, counterclockwise absolute orientation, two-membered flagellar rootlets replacing the spline\*/multilayered structure rootlet system, and basal bodies always showing a parallel orientation to the axis of motion. The prasinophyte *Pyramimonas* is the closest living approximation to this assemblage of ancestral attributes. As regards the class Charophyceae, however, the existing knowledge seems inadequate to warrant any clear or convincing scheme regarding its origin or phylogeny.

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\*Some workers term this structure as microtubular band.



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# 8 Cell Division and Cytokinesis

## GREEN ALGAE

Much of our knowledge about cell division and cytokinesis in algae is based on researches on the fine structure of several green algae during the last two decades.

Nuclear division in many unicellular flagellates is immediately followed by cleavage of the mother cell into two daughter cells. In *Chlamydomonas*, cleavage is always longitudinal and the circumferential furrow usually grows through the cell unevenly; this growth is faster from the end occupied by the basal bodies. Before the onset of prophase, the basal bodies replicate (Goodenough, 1970). At least two distinct sets of microtubules are involved in the complicated cleavage apparatus in *C. reinhardtii*. After telophase, a network of endoplasmic reticulum interconnects the daughter nuclei. An array of parallel internuclear microtubules appears and these are oriented at right angles to the spindle axis and are confined to the cytoplasm immediately between the nuclei. During early cleavage in *C. reinhardtii*, the cleavage furrow grows inward between daughter nuclei, and two sets of microtubules are coplanar with the cleavage furrow, the cleavage microtubules, and the internuclear microtubules. With the progress of cleavage, the cleavage furrow grows inward all around the cell, and the chloroplast also starts furrowing in the plane of cell cleavage, thus partitioning the pyrenoid into daughter cells. At early cleavage, the nuclei are situated peripherally but at late cleavage, they move centrally and this is accompanied by the rotation of the cytoplasm through 90°. The interesting point to note is that even a primitive alga like *Chlamydomonas* utilizes at least two sets of microtubules in its cytokinesis. In this alga, cytokinesis and release of daughter cells lag behind karyokinesis, but no nucleus contains more than the diploid quantity of DNA. This means that daughter cell formation occurs by doubling of the nuclear DNA followed by karyokinesis, in a repeated sequence. During the formation of daughter cells, the parental cell becomes multinucleate temporarily (Coleman, 1982).

When the alga is grown on a 12 hr light : 12 hr dark cycle, its nuclear DNA starts to replicate just before nuclear division near the end of the light period. In contrast, the replication of its chloroplast DNA begins much earlier, in the first half of the light period. Recent biochemical studies have indicated the presence of about 50–100 plastid DNA genomes comprising about 12–15% of the total cellular DNA (Turmel *et al.*, 1980). Coleman (1978) made cytological observations and reported that individual cells contain up to 12–15 localized areas of DNA in the chloroplast. It is not known how the cell regulates the number and distribution of genomes in the plastid in coordination with its nuclear cycle.

In *Tetraspora*, the basal bodies do not seem to replicate before prophase but other stages of cytokinesis resemble those in *Chlamydomonas*.

Mitosis and cytokinesis in *Chlorella* involve a cleavage furrow, a metaphase band of microtubules, a perinuclear envelope, and persistent centrioles associated with spindle and the cleavage apparatus (Atkinson *et al.*, 1971). Persistent centrioles also occur in *Scenedesmus*, usually located close to the nucleus.



A complex series of cytoplasmic cleavages is characteristic of *Hydrodictyon*. These cleavages cut up the multinucleate cytoplasm into thousands of minute uninucleate swimmers which may become zoospores or gametes. The cleavage microtubules are oriented in the plane of cytokinesis.

Cell division and cytokinesis in *Klebsormidium* have been studied by Floyd *et al.* (1972) and Pickett-Heaps (1972). Division commences with the cleavage of the chloroplast and pyrenoid. During prophase and metaphase, the nucleus remains appressed to the chloroplast, though in a deformed state. Wall microtubules disappear. The centrioles separate, the spindle poles are established at prophase, and many microtubules radiate from the poles. These microtubules cover the elongating, spindle-shaped nucleus. The nucleolus disperses. The chromosomes appear. The nuclear envelope breaks up and disperses. The metaphase spindle is elongated and open. Chromosome separation during anaphase involves two interesting phenomena, viz., (1) the constancy of the chromosome-to-pole distance and (2) the appearance of two vacuoles in the interzone between daughter chromosomes, followed by their fusion and growth at the expense of the terminal vacuole. At telophase, the daughter nuclei remain widely separated, and cytokinesis as well as interzonal microtubules persist between these remotely separated nuclei. Nuclear envelope reforms but not all chromosomal microtubules disperse. Unlike the green algae described in the above paragraphs, in *Klebsormidium*, cytokinesis occurs by the ingrowth of an annular furrow of the plasmalemma. This ingrowing furrow appears at metaphase and finally cuts the interzonal vacuole, mitochondria, and the remains of interzonal microtubules. Eventually, the persistent microtubules near the centrioles disperse, and daughter cells are formed.

In *Coleochaete scutata*, the daughter nuclei are widely separated at telophase, and a phragmoplast is used in cross wall formation. In a phragmoplast, the cytokinetic microtubules are not oriented in the plane of cytokinesis but rather are oriented perpendicular to this plane. The longitudinally-oriented microtubules proliferate between the daughter nuclei and the cross wall is formed by fusions of small vesicles.

Unique, extraordinary events are involved in the interesting process of cell division in *Oedogonium* and *Bulbochaete*, leading to the formation of cap cells. In these genera, the newly formed daughter cells expand abruptly and rapidly and an internal, subapical ring is involved in cell division. The spindle is closed. Fine filaments (about 8 nm in diameter) are attached to kinetochores from prophase through anaphase. At prometaphase, microtubules penetrate the nucleus and in some manner seem to interact with the microfilaments. Several microtubules associate laterally with kinetochores (Schibler and Pickett-Heaps, 1980). After telophase, the elongated spindle collapses. Between the daughter nuclei, a transversely disposed system of microtubules is laid down and divides the cell into two. The wall of the mother cell ruptures at the apical ring and at this zone, rapid elongation and growth of the new wall occur. The division ring of *Bulbochaete hiloensis* is somewhat abnormal in the sense that part of the ring is located in the middle of the end wall of the cell.

In male cells of *Sphaeroplea annulina*, multiple mitoses, each associated with phycoplast microtubules, are followed by cytoplasmic cleavage delineated by microtubules, and many male gametes are formed. These gametes remain within a vacuolar envelope until maturity. At maturity, the nucleus decreases in size, the chromatin condenses, and the chloroplast thylakoids, endoplasmic reticulum, and Golgi apparatus are lost (Caceres and Robinson, 1981). The apical zone of



flagellar insertion consists of an apical cone and fibrous connections which lie distal to the basal bodies.

During mitosis, *S. annulina* shows a collapsed spindle at telophase. Its motile cells have anteriorly inserted flagella with the basal bodies associated with four cruciate bands of microtubules. These characters suggest that this alga belongs to the class Chlorophyceae *sensu* Stewart and Mattox (1975) and not to the Charophyceae.

*Sphaeroplea* spermatogenesis is in many respects similar to the events happening during zoosporogenesis in *Hydrodictyon*. These include: (1) the proliferation of phycoplast microtubules in the cleavage furrow and (2) the formation of a vacuolar envelope separating the swimmers from the vacuolar sap during their maturation. However, unlike *Hydrodictyon*, the vacuolar envelope in *Sphaeroplea* is oriented at right angles to the cell axis since its cytoplasm is arranged in rings. Further, the gametes of *Sphaeroplea* show a much greater reduction of organelles as compared to the zoospores and gametes of Chlorococcalean algae, and the number of microtubules in flagellar roots in the two cases also differs. *Sphaeroplea* also resembles *Oedogonium* in the following respects: (1) the flagellar rootlets have an equal number of microtubules in both genera, (2) both are oogamous, have phycoplast, and their modes of cytokinesis and cell wall formation are identical, (3) an unusual type of pyrenoid having large cytoplasmic invaginations occurs in both genera, and (4) the flagellar apparatuses of the two genera are broadly similar (Caceres and Robinson, 1981). These considerations have prompted Caceres and Robinson to propose that *Sphaeroplea* may best be placed in a separate order Sphaeropleales in the class Chlorophyceae.

Fowke and Pickett-Heaps (1969) and Jordan and Godward (1969) have studied cell division and cytokinesis in *Spirogyra*. At preprophase, the nucleus enlarges and a thickening of certain cytoplasmic strands, which cover the nucleus, occurs. These strands connect the nucleus with the chloroplast and they contain many microtubules. The nucleus contains a central spherical and dense nucleolus. Endoplasmic reticulum orients along the future spindle axis. Shortly before prophase, diffuse chromosomes appear and start condensing in and around the nucleolus. The nuclear envelope develops gaps during metaphase and through these gaps some microtubules penetrate into the nucleus. Spindle elongates further at anaphase and the nuclear envelope completely disperses by this stage. The spindle microtubules are distributed mainly in a cylindrical array, enclosing the two groups of chromosomes. During telophase, new nuclear envelopes are synthesized and new nucleoli are formed (around the remnants of the old nucleoli). The persistent microtubules lying between the daughter nuclei participate in cytokinesis which occurs by an ingrowing cleavage furrow that impinges upon the cylindrical mass of interzonal cytoplasm having many microtubules. Phragmoplast is organized at inner edges of cleavage furrow. Cytokinesis is completed by the formation of a cell plate.

In contrast to the persistent nucleoli in *Spirogyra*, the prominent interphase nucleolus disperses completely in *Zygnema*.

Both *Spirogyra* and *Microspora* use furrowing as well as vesicles for the formation of their cross walls and yet their microtubular systems are quite unlike since *Microspora* forms a typical phycoplast, whereas *Spirogyra* has a rudimentary phragmoplast.

The processes of mitosis and cytokinesis in desmids have been studied by several workers, especially in *Micrasterias* and *Closterium*. In *Micrasterias*, whenever the cells divide, the septum balloons out, attaining a perfect shape of



the older semi-cell. A girdle of primary wall material is first laid down just beneath the secondary wall at the isthmus. It thickens and widens, thereby allowing the semi-cells to detach and separate from one another. A septum is then formed around the circumference of the girdle at its midpoint (Lacalli, 1981). Inward growth of the septum causes separation of the cytoplasms of the daughter cells by telophase. The septum splits progressively, resulting in two flattened hemispherical bulges. The new primary wall maintains its symmetry around the polar axis up to this stage but later on it produces a twofold symmetry corresponding to that of the parent semi-cell. The new semi-cells successively pass through 3-lobed, 5-lobed, 9-lobed, and 7-lobed stages, some of which may be quite transitory. The changes in symmetry occur in discrete steps and symmetry appears to be predetermined by the time of cytokinesis. It seems that changes in symmetry and ornamentation are not necessarily caused by genetic phenomena (see Pickett-Heaps, 1975).

One very striking feature of desmid development is that the same region of the developing semi-cell can produce ingrowths or outgrowths depending on species and conditions. Thus, a girdle and septum seem to be established during cytokinesis in most or all placoderm desmids. During conjugation, an identical girdle is formed at the isthmus, followed by outgrowths of a lobe-like conjugation papilla (Lacalli, 1981).

Whereas numerous bundles of microtubules can be seen in *Micrasterias* immediately after cell division, *Closterium* seems to lack any association of microtubules with the ingrowing annular furrow (Kiermayer, 1968; Pickett-Heaps and Fowke, 1970). However, *Closterium* does possess a system of transversely-oriented microtubules lying near its cell wall, after the completion of the cell division. In *Closterium*, the system of microtubules forms in the cytoplasm near or at the spindle poles. These microtubules are also involved in the movement of the daughter nuclei along each semi-cell.

In most plant cells, microtubules are important in the formation of the fibrillar cell walls, but many desmids can synthesize elaborate and complex fibrillar walls without involving microtubules. Likewise, centrioles play an important role in the division of animal cells but many plant cells can divide quite well without centrioles.

The prophase and metaphase stages in antheridia of *Chara preissii* (= *C. fibrosa*) involve acentric and open spindles (Pickett-Heaps, 1967, 1975). Some dense nucleolar remnants remain attached to chromosomes, even through later anaphase. Like higher plants, the spindle fibres aggregate at the interzone before a typical cell plate phragmoplast is formed. At telophase, the nuclei remain far apart. The cortical cells arise by asymmetrical divisions. The phragmoplast tubules in *Chara* are not as numerous as in higher plants. Centrioles are absent.

## CYTOLOGICAL CLASSIFICATION

Researches on green algae carried out by Pickett-Heaps, Stewart, Mattox, and other cytologists have highlighted the importance of phycoplast and phragmoplast in cytological classification of these algae.

Four general patterns of cell division have been realized in green algae: (1) no spindle is formed, daughter nuclei lie close together at telophase, a phycoplast (i.e., transversely arranged microtubules oriented in the plane of cytokinesis) is organized, and cytokinesis occurs by cell plate formation, (2) same as in (1) but cytokinesis occurs by furrowing, (3) spindle is persistent, nuclei lie away from



each other, phragmoplast (i.e., microtubules are longitudinally arranged, being oriented perpendicular to the plane of cytokinesis) is formed, and cytokinesis involves cell plate formation, and (4) same as in (3) but cytokinesis is by furrowing.

The type (1) is exemplified by *Oedogonium*, type (2) by *Tetraedron*, type (3) by *Chara*, and type (4) by *Klebsormidium*.

Based on studies of these different types of cell division and cytokinesis, Pickett-Heaps (1975) and Stewart and Mattox (1975) proposed the grouping of green algae into two distinct classes, the Chlorophyceae and the Charophyceae.

The Chlorophyceae forms swimmers whose flagella are anteriorly inserted and whose flagellar basal bodies are associated with four or more cruciately-arranged bands of microtubules. Phycoplast formation is typical of this class. Stewart and Mattox include the following orders in the class: Volvocales, Chlorococcales, Microsporales, Ulvales, Chaetophorales, and Oedogoniales.

In contrast, the Charophyceae produces motile cells whose flagella are somewhat laterally attached and whose basal bodies are associated with a single band of microtubules. Unlike the narrow bands of microtubules in the Chlorophyceae, the single band in the Charophyceae is quite broad. In the Charophyceae, mitotic spindle is open and a phragmoplast proliferating from a persistent interzonal spindle is involved in cytokinesis. As thus delimited, the Charophyceae includes the orders Klebsormidiales, Zygnematales, Coleochaetales, and Charales. Another characteristic of the Charophyceae is that the cells have the enzyme glycollate oxidase. In contrast, cells of the Chlorophyceae lack this enzyme.

On the basis of their observations on the cytology of several green algae, Stewart *et al.* (1973) have proposed that the large order Chaetophorales *sensu* Fritsch should be split up into four smaller orders, viz., Ulotrichales, Microsporales, Chaetophorales, and Coleochaetales (Table XIII). Of these, the Ulotrichales seem to be cytologically somewhat different from the remaining three orders.

More recent work on the cytobiology of green algae has led to further modification of their classification. To the two classes Chlorophyceae and Charophyceae, Stewart and Mattox (1978) have added one more, naming it Ulvaphyceae. The proposal to classify the Chlorophyta into these three classes was largely based on ultrastructural studies of such genera as *Trichosarcina*, *Monostroma*, *Ulvopsis*, *Ulva*, *Ulothrix*, and *Pseudendoclonium*. The proposal has received support from later work of Melkonian (1980) and Sluiman *et al.* (1980). Unlike the Charophyceae, the Ulvaphycean zoospores lack the unilateral multilayered structure. However, like the Charophyceae, the zoospores of the Ulvaphyceae sometime possess body scales.

Some general characters of the three classes of green algae are listed in Table XIV.

More recently, however, Lokhorst and Star (1983) and Bakker and Lokhorst (1984) have disputed the validity and distinction of the class Ulvaphyceae. These workers feel that detailed, critical studies of the fine structure of flagellar apparatus of such genera as *Draparnaldia*, *Cylindrocapsa*, *Fritschiella*, *Schizomeris*, *Stigeoclonium*, and *Uronema*, together with other features like the similar basic type of the mitotic and cytokinetic mechanism in these algae, fail to furnish any justification for the creation of the class Ulvaphyceae. Thus, according to Bakker and Lokhorst (1984), it is better to follow Stewart and Mattox (1975) who divided the green algae into only two classes, viz., Chlorophyceae and Charophyceae. On the other hand, Sluiman (1985) recognizes the Ulvophyceae and circumscribes it as follows: Motile cells, when bi- or quadriflagellate, are provided with



Table XIII Comparative cytological characters of the four orders proposed by Stewart *et al.* (1973)

Property	Ulotrichales	Microsporales	Chaetophorales	Coleochaetales
Mode of anaphase movement of chromosomes	Spindle elongation	Shortening of chromosome-to-pole distance	Shortening of chromosome-to-spindle distance	No significant change in chromosome-to-pole distance
Status of spindle at telophase	Interzonal spindle, tubules persist	Non-persistent	Non-persistent	
Mode of cytokinesis	Cleavage or furrowing	Furrowing	Cell plate formation	Cell plate formation
Participation of microtubules in cytokinesis	Nil* (no phycoc- or phragmoplast)	Phycoplast present	Phycoplast present	Phragmoplast present
Plasmodesmata	Absent	Absent	Present	Present

\*Phycoplast formation has since been reported in some genera.



Table XIV Some general features of three classes of green algae (after Sluiman *et al.*, 1980)

Character	Chlorophyceae	Ulvophyceae	Charophyceae
Cytokinesis	Phycoplast present	Phycoplast absent; furrow precocious	Phragmoplast present; furrow with persistent spindle
Zoospores and flagellated gametes			
Flagellar root system	Cruciate	Cruciate	Unilateral
Body scales	Absent	May or may not be present	May or may not be present
Basal body connecting			
Elements	Striated	Mainly non-striated	Striated
Terminal cap	Absent	Present	Absent
Rhizoplast	Absent	Present	Absent



apically attached flagella that do not arise from a pit. Scales, if present, occur in one layer only and are not on flagellar plasma membrane. Basal bodies 1 and 2 form a V- or L-shaped pair at angles between  $90^\circ$  and  $220^\circ$  and are slightly displaced laterally in a counterclockwise direction. During vegetative cell division, mitotic spindles are enclosed by a persisting nuclear membrane, and spindle poles are associated with centrioles.

### PRASINOPHYCEAE

The nucleus is commonly located between the basal bodies and the base of the chloroplast, adjacent to the pyrenoid. The rhizoplast generally lies along the nuclear surface and may be attached both to it and the cell membrane. The polarization of the nucleus toward the basal bodies differs from the position in the green algal flagellates where the nucleus is commonly centrally located.

Stewart *et al.* (1974) reported that in *Platymonas subcordiformis* the interzonal spindle collapses at telophase and a system of transversely-oriented microtubules develops between the daughter nuclei. These microtubules constitute the phycoplast. Later studies on *Pedinomonas* have shown it to have a persistent interzonal spindle (Pickett-Heaps and Ott, 1974). Thus, *Pedinomonas* differs from *Platymonas*. A third prasinophyte, viz., *Pyramimonas*, seems to lack a phycoplast and its spindle is open. During its cytokinesis, a furrow grows between widely-separated daughter nuclei. The occurrence of three different patterns of cell division and cytokinesis in three genera of this class prompted Pickett-Heaps (1975) to suggest that the class Prasinophyceae does not include phylogenetically related organisms but is heterogeneous.

The mitotic process in the uniflagellate scaly alga *Mantoniella squamata* is unlike that in other prasinophytes. The cell enlarges and its single chloroplast and pyrenoid divide. The single Golgi body, the single mitochondrion, and the two basal bodies also undergo division. The replicated basal bodies and flagella separate as the nucleus enlarges. A Golgi body is located at each spindle pole. The basal bodies lie within invaginations of the nucleus antapical to the poles (see Barlow, 1977). Barlow's work and also that of other recent workers supports the view of Pickett-Heaps as to the heterogeneity of the class Prasinophyceae.

### RHODOPHYCEAE

Some work has been done on mitosis and cytokinesis in red algae. These organisms have many features reminiscent of animal cells. They synthesize diverse secretory and metabolic products located in membrane-bound vesicles. They show complex interactions between Golgi bodies and endoplasmic reticulum. They also seem to display nuclear-cytoplasmic interactions similar to those found in embryonic and rapidly differentiating animal cells. Their cells have microtubules but lack centrioles and flagella. They have pit connections between adjoining cells. Pit connections are lenticular plugs with several-layered caps. These connections occupy a central position in the septa between adjacent cells. Open communications do not occur between cells of red algae. The liberation of spermatia, tetraspores, and carpospores occurs via apertures from which pit connections have been displaced, in the walls of the mother alga (Duckett and Peel, 1978). Another common and widespread phenomenon in red algae is the successive development of new structures within existing walls, e.g., on spermatial branches.

Red algal cell walls characteristically resemble the cell walls of higher fungi in many ways. The Rhodophycean pit connections are very similar to septal plugs



Table XV Comparison of some ultrastructural aspects of cell division in three genera of red algae (after Scott, 1983)

Character	<i>Porphyridium</i>	<i>Batrachospermum</i>	<i>Polysiphonia</i>
Nuclei per cell	1	1	Many
Bipartite nuclear associated organelles	Large oblong proximal granule and small distal discoidal part; at prometaphase, proximal part breaks up.	Small ring within a larger ring; behaviour at prometaphase not known	Superimposed equal sized proximal and distal rings; at prometaphase, proximal and distal rings disjoin
Prometaphase microtubule containing cytoplasmic channels	Absent	Present	Absent
Kinetochore	Small, simple	Structure unknown	Large, three-layered
Microtubules per kinetochore	1	?	Several
Perinuclear ER	Absent	Present	Present
Nuclear envelope condition At late prophase	One nuclear pocket present at each pole	Two nuclear pockets present at each pole	One protrusion of nuclear envelope present at each pole
At metaphase	Intact, except for single, large gap at each pole	Intact, except for single, large gap at each pole	Intact, except for numerous, small fenestrations at each pole
Cytokinetic apparatus	Cleavage furrows grow through enlarged chloroplast, separating daughter nuclei	Cleavage furrows grow through central vacuole, separating daughter nuclei	Cleavage furrows grow through central vacuole, separating daughter nuclei

In all three genera, a typical metaphase chromosomal plate is present, chromosomal and non-chromosomal spindle microtubules are present, and the fragments of dispersing nucleoli are retained in daughter nuclei.



of fungi. The popular view of the red algal ancestry of higher fungi is strongly supported by the production of apoplastic cells by a process which completely excludes all plastid material from specific cells at cytokinesis (Duckett and Peel, 1978).

In *Batrachospermum*, the thallus is composed of three major cell types which develop as a consequence of three different modes of cell division. Apical cells have distally located nucleus. When this divides, one remains distal, whereas the other migrates to the proximal end of the cell. When a pericentral cell develops from an axial one, the nucleus remains within the latter, migrates near the junction of the two cells and there divides; one daughter nucleus then migrates to the lateral initial, whereas the other moves back into the axial cell. The nucleus remains in the mother cell when a pleuridial cell initial grows and expands.

A new type of vegetative pit connection in the main axis of *B. sirodotii* has been described by Aghajanian (1977). It is made of a rivet-like plug which occludes a pore in the pit ring. This pit plug seems to be derived from the endoplasmic reticulum and Golgi apparatus.

A direct involvement of endoplasmic reticulum in cytokinesis is uncommon but has been reported in the red alga *Harveyella* (Kugrens and Koslowsky, 1981). The tetrasporocytes of this Cryptonemalian alga exhibit this unique kind of cytokinesis.

In most Florideophyceae, cytokinesis involves a centripetal wall deposition and, usually, the formation of a septal plug in the aperture of an incompletely formed septum (Lee, 1971). But no septal plug is formed during tetrasporocyte cleavage where septum formation is complete. In *Harveyella*, cytokinesis occurs immediately after four post-meiotic nuclei are formed, and is thought to result from the coalescence of endoplasmic cisternae. This kind of cisternal coalescence leads to the formation of a double-membraned tetrahedral cleavage channel that lacks a wall. Some membranes of the cleavage channel fuse with the existing tetrasporocyte plasmalemma, delimiting four tetraspores. The wall material is then secreted in an unusual manner in that no dictyosomes are involved during the first wall secretion stage at all; the dictyosomes only contribute to secondary wall deposition.

Table XV compares some fine structural aspects of nuclear and cell division in *Porphyridium* (Bangiophyceae) with two representative genera of the Florideophyceae.

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This monograph attempts to bring together some puzzling questions of perennial interest to cytophycologists and discusses them in the light of the explosion in the knowledge of the fine structure of diverse algae and their organelles. The bearing of recent advances in algal cytobiology on several phylogenetic and evolutionary problems is assessed with special reference to the work on flagella, cell walls, nuclear cytology, cytokinesis, eyespots, and chloroplasts. The views of early botanists, such as Bower and Fritsch, on the algal ancestry of higher plants are analyzed by taking into account phycoplast versus phragmoplast formation and recent contributions on many green algae. In addition to the eukaryotic algae, which are considered in sufficient detail, the prokaryotic cyanobacteria and *Prochloron* are briefly described so as to review the subject in a holistic perspective.

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